

This Page Is Inserted by IFW Operations
and is not a part of the Official Record

BEST AVAILABLE IMAGES

Defective images within this document are accurate representations of the original documents submitted by the applicant.

Defects in the images may include (but are not limited to):

- BLACK BORDERS
- TEXT CUT OFF AT TOP, BOTTOM OR SIDES
- FADED TEXT
- ILLEGIBLE TEXT
- SKEWED/SLANTED IMAGES
- COLORED PHOTOS
- BLACK OR VERY BLACK AND WHITE DARK PHOTOS
- GRAY SCALE DOCUMENTS

IMAGES ARE BEST AVAILABLE COPY.

**As rescanning documents *will not* correct images,
please do not report the images to the
Image Problem Mailbox.**

THIS PAGE BLANK (USPTO)

PCT

WORLD INTELLECTUAL PROPERTY ORGANIZATION
International Bureau



INTERNATIONAL APPLICATION PUBLISHED UNDER THE PATENT COOPERATION TREATY (PCT)

<p>(51) International Patent Classification ⁶ : C12N 15/12, A61K 39/395, C07K 14/47, G01N 33/68, 33/566, 33/53, A61K 38/17, 48/00 // C12Q 1/68</p>	<p>A1</p>	<p>(11) International Publication Number: WO 96/14411 (43) International Publication Date: 17 May 1996 (17.05.96)</p>
<p>(21) International Application Number: PCT/US95/14024 (22) International Filing Date: 27 October 1995 (27.10.95) (30) Priority Data: 08/336,235 3 November 1994 (03.11.94) US (71) Applicant: THE REGENTS OF THE UNIVERSITY OF CALIFORNIA [US/US]; 22nd floor, 300 Lakeside Drive, Oakland, CA 94612 (US). (72) Inventors: NGUYEN, Thai, D.; 17 Central Valley Drive, Mill Valley, CA 94941 (US). POLANSKY, Jon, R.; 15 Stanton Way, Mill Valley, CA 94941 (US). HUANG, Weidong; 42 Behr Avenue, San Francisco, CA 94131 (US). (74) Agents: FREED, Joel, M. et al.; Howrey & Simon, 1299 Pennsylvania Avenue, N.W., Washington, DC 20004-2402 (US).</p>		<p>(81) Designated States: AM, AT, AU, BB, BG, BR, BY, CA, CH, CN, CZ, DE, DK, EE, ES, FI, GB, GE, HU, IS, JP, KE, KG, KP, KR, KZ, LK, LR, LS, LT, LU, LV, MD, MG, MK, MN, MW, MX, NO, NZ, PL, PT, RO, RU, SD, SE, SG, SI, SK, TJ, TM, TT, UA, UG, UZ, VN, European patent (AT, BE, CH, DE, DK, ES, FR, GB, GR, IE, IT, LU, MC, NL, PT, SE), OAPI patent (BF, BJ, CF, CG, CI, CM, GA, GN, ML, MR, NE, SN, TD, TG), ARIPO patent (KE, LS, MW, SD, SZ, UG).</p> <p>Published <i>With international search report. Before the expiration of the time limit for amending the claims and to be republished in the event of the receipt of amendments.</i></p>
<p>(54) Title: METHODS FOR THE DIAGNOSIS OF GLAUCOMA</p> <p>(57) Abstract</p> <p>A glucocorticoid-induced protein, TIGR, that is produced by cells of the trabecular meshwork can be used to diagnose glaucoma. The TIGR protein, anti-TIGR antibodies, and TIGR encoding sequences also provide a diagnostic for glaucoma and its related diseases.</p>		

FOR THE PURPOSES OF INFORMATION ONLY

Codes used to identify States party to the PCT on the front pages of pamphlets publishing international applications under the PCT.

AT	Austria	GB	United Kingdom	MR	Mauritania
AU	Australia	GE	Georgia	MW	Malawi
BB	Barbados	GN	Guinea	NE	Niger
BE	Belgium	GR	Greece	NL	Netherlands
BF	Burkina Faso	HU	Hungary	NO	Norway
BG	Bulgaria	IE	Ireland	NZ	New Zealand
BJ	Benin	IT	Italy	PL	Poland
BR	Brazil	JP	Japan	PT	Portugal
BY	Belarus	KE	Kenya	RO	Romania
CA	Canada	KG	Kyrgyzstan	RU	Russian Federation
CF	Central African Republic	KP	Democratic People's Republic of Korea	SD	Sudan
CG	Congo	KR	Republic of Korea	SE	Sweden
CH	Switzerland	KZ	Kazakhstan	SI	Slovenia
CI	Côte d'Ivoire	LI	Liechtenstein	SK	Slovakia
CM	Cameroon	LK	Sri Lanka	SN	Senegal
CN	China	LU	Luxembourg	TD	Chad
CS	Czechoslovakia	LV	Latvia	TG	Togo
CZ	Czech Republic	MC	Monaco	TJ	Tajikistan
DE	Germany	MD	Republic of Moldova	TT	Trinidad and Tobago
DK	Denmark	MG	Madagascar	UA	Ukraine
ES	Spain	ML	Mali	US	United States of America
FI	Finland	MN	Mongolia	UZ	Uzbekistan
FR	France			VN	Viet Nam
GA	Gabon				

TITLE OF THE INVENTION:

5

METHODS FOR THE DIAGNOSIS OF GLAUCOMA**FIELD OF THE INVENTION:**

10 The present invention is in the fields of diagnostics, and concerns methods and reagents for diagnosing glaucoma and related disorders. This invention was supported with Government funds (NIH EY02477 and NIH EY 08905-02). The Government has certain rights in this invention.

BACKGROUND OF THE INVENTION:

15 "Glaucomas" are a group of debilitating eye diseases that are the leading cause of preventable blindness in the United States and other developed nations. Primary Open Angle Glaucoma ("POAG") is the most common form of glaucoma. The disease is characterized by the degeneration of the trabecular meshwork, leading to obstruction of the normal ability of aqueous humor to leave the eye without closure of the space (e.g., the "angle")
20 between the iris and cornea (see, Vaughan, D. *et al.*, In: *General Ophthalmology*, Appleton & Lange, Norwalk, CT, pp. 213-230 (1992)). A characteristic of such obstruction in this disease is an increased intraocular pressure ("IOP"), resulting in progressive visual loss and blindness if not treated appropriately and in a timely fashion.

25 The disease is estimated to affect between 0.4% and 3.3% of all adults over 40 years old (Leske, M.C. *et al.*, *Amer. J. Epidemiol.* 113:1843-1846 (1986); Bengtsson, B., *Br. J. Ophthalmol.* 73:483-487 (1989); Strong, N.P., *Ophthalm. Physiol. Opt.* 12:3-7 (1992)). Moreover, the prevalence of the disease rises with age to over 6% of those 75 years or older (Strong, N.P., *Ophthalm. Physiol. Opt.* 12:3-7
30 (1992)).

A link between the IOP response of patients to glucocorticoids and the disease of POAG has long been suspected. While only 5% of the normal population shows a high IOP increase (16 mm Hg) to topical glucocorticoid

testing, over 90% of patients with POAG show this response. In addition, an open angle glaucoma may be induced by exposure to glucocorticoids. This observation has suggested that an increased or abnormal glucocorticoid response in trabecular cells may be involved in POAG (Zhan, G.L. *et al.*, *Exper. Eye Res.* 54:211-218 (1992); Yun, A.J. *et al.*, *Invest. Ophthalmol. Vis. Sci.* 30:2012-2022 (1989); Clark, A.F., *Exper. Eye Res.* 55:265 (1992); Klemetti, A., *Acta Ophthalmol.* 68:29-33 (1990); Knepper, P.A., U.S. Patent No. 4,617,299).

The ability of glucocorticoids to induce a glaucoma-like condition has led to efforts to identify genes or gene products that would be induced by the cells of the trabecular meshwork in response to glucocorticoids (Polansky, J.R. *et al.*, In: *Glaucoma Update IV*, Springer-Verlag, Berlin, pp. 20-29 (1991)). Initial efforts using short-term exposure to dexamethasone revealed only changes in specific protein synthesis. Extended exposure to relatively high levels of dexamethasone was, however, found to induce the expresion of related 66 kD and 55 kD proteins that could be visualized by gel electrophoresis (Polansky, J.R. *et al.*, In: *Glaucoma Update IV*, Springer-Verlag, Berlin, pp.20-29 (1991)). The induction kinetics of these proteins as well as their dose response characteristics were similar to the kinetics those that were required for steroid-induced IOP elevation in human subjects (Polansky, J.R. *et al.*, In: *Glaucoma Update IV*, Springer-Verlag, Berlin, pp. 20-29 (1991)). Problems of aggregation and apparent instability or loss of protein in the purification process were obstacles in obtaining a direct protein sequence.

Because increased IOP is a readily measurable characteristic of glaucoma, the diagnosis of the disease is largely screened for by measuring intraocular pressure (tonometry) (Strong, N.P., *Ophthalm. Physiol. Opt.* 12:3-7 (1992), Greve, M. *et al.*, *Can. J. Ophthalmol.* 28:201- 206 (1993)). Unfortunately, because glaucomatous and normal pressure ranges overlap, such methods are of limited value unless multiple readings are obtained (Hitchings, R.A., *Br. J. Ophthalmol.* 77:326 (1993); Tuck, M.W. *et al.*, *Ophthalm. Physiol. Opt.* 13:227-232 (1993); Vaughan, D. *et al.*, In: *General Ophthalmology*, Appleton & Lange, Norwalk, CT, pp. 213-230 (1992); Vernon, S.A., *Eye* 7:134-137 (1993)). For this reason, additional methods, such as direct examination of the optic disk and determination of the extent of a patient's visual field loss are often conducted to improve the accuracy of diagnosis (Greve, M. *et al.*, *Can. J. Ophthalmol.* 28:201- 206 (1993)).

- 3 -

In view of the importance of glaucoma, and the at least partial inadequacies of prior methods of diagnosis, it would be desirable to have an improved, more accurate method for diagnosing glaucoma. The present invention provides such improved diagnostic agents and methods.

5 **SUMMARY OF THE INVENTION:**

10 The invention concerns a novel peptide sequence discovered to be highly induced by glucocorticoids in the endothelial lining cells of the human trabecular meshwork. The cDNA for this protein, the protein itself, molecules that bind to it, and nucleic acid molecules that encode it, provide improved
15 methods and reagents for diagnosing glaucoma and related disorders, as well as for diagnosing other diseases or conditions, such as cardiovascular, immunological, or other diseases or conditions that affect the expression or activity of the protein. Indeed, the molecules of the present invention may be used to diagnose diseases or conditions which are characterized by alterations
20 in the expression of extracellular proteins. In addition, due to its cellular functions and DNA binding properties, the molecules of the present invention may be used to diagnose diseases or conditions which are characterized those functions.

20 The clone was termed "II.2" during the procedures of its isolation and was subsequently renamed "TIGR" ("Trabecular Meshwork Induced Glucocorticoid Response") protein.

In detail, the invention provides a substantially purified TIGR protein having the sequence of SEQ ID NO:1 residues 1-497 or 15-197.

25 The invention further provides a nucleic acid molecule that encodes a TIGR protein, especially a nucleic acid molecule that comprises the sequence of SEQ ID NO:2 or SEQ ID NO:3.

The invention also provides an antibody capable of specifically binding to a TIGR protein.

30 The invention also provides a method for diagnosing glaucoma in a patient which comprises determining whether the amount of a TIGR protein present in the trabecular meshwork of an eye of the patient exceeds the amount of that TIGR protein present in the trabecular meshwork of an eye of an individual who is not suffering from glaucoma, wherein the detection of an excessive amount of the TIGR protein is indicative of glaucoma.

- 4 -

5 The invention also provides a method for quantitatively or qualitatively determining the amount of a TIGR protein present in the trabecular meshwork of an eye of an individual, and determining whether that amount exceeds the amount of that TIGR protein present in the trabecular meshwork of an eye of an individual who is not suffering from glaucoma, wherein the detection of an excessive amount of the TIGR protein is indicative of glaucoma.

BRIEF DESCRIPTION OF THE FIGURE:

Figures 1A-1D provide the amino acid sequence of the TIGR protein and the sequence of the cDNA that encodes the TIGR protein.

10 DETAILED DESCRIPTION OF THE INVENTION:

I. Overview of the Invention

15 As indicated above, the trabecular meshwork has been proposed to play an important role in the normal flow of the aqueous, and has been presumed to be the major site of outflow resistance in glaucomatous eyes. Human trabecular meshwork (HTM) cells are endothelial like cells which line the outflow channels by which aqueous humor exits the eye; altered synthetic function of the cells may involve in the pathogenesis of steroid glaucoma and other types of glaucoma. Sustained steroid treatment of these cells are interesting because it showed major difference was observed when compared to 1-2 day glucocorticoid (GC) exposure, which appears relevant to the clinical onset of steroid glaucoma (1-6 weeks).

20 Despite decades of research, prior to the present invention, the molecular basis for glaucoma had not been determined (Snyder, R.W. *et al.*, *Exper. Eye Res.* 57:461-468 (1993); Wiggs, J.L. *et al.*, *Genomics* 21:299-303 (1994)).

25 Although trabecular meshwork cells had been found to induce specific proteins in response to glucocorticoids (see, Polansky, J.R., In: "*Schriftenreihe de Adademie der Wissenschaften und der Literatur, Mainz*," 307-318 (1993)), efforts to purify the expressed protein were encumbered by insolubility and other problems. Nguyen, T.D. *et al.* (In: "*Schriftenreihe de Adademie der Wissenschaften und der Literatur, Mainz*," 331-343 (1993), herein incorporated by reference) used a molecular cloning approach to isolate a highly induced mRNA species from glucocorticoid-induced human trabecular cells. The mRNA exhibited a

30

- 5 -

time course of induction that was similar to the glucocorticoid-induced proteins. The clone was designated "II.2"

5 The present invention stems in part from the recognition that the isolated II.2 clone encodes a novel secretory protein (termed "Trabecular Meshwork Induced Glucocorticoid Response" protein or "TIGR") that is induced in cells of the trabecular meshwork upon exposure to glucocorticoids. It has been proposed that this protein may become deposited in the extracellular spaces of the trabecular meshwork and bind to the surface of the endothelial cells that line the trabecular meshwork, thus causing a decrease in aqueous flow. Quantitative dot blot analysis and PCR evaluations have shown that the TIGR mRNA exhibits a progressive induction with time whereas other known GC-inductions from other systems and found in HTM cells (metallothionein, alpha-1 acid glycoprotein and alpha-1 antichymotrypsin) reached maximum level at one day or earlier. Of particular interest, the induction level of this clone was very high (4-6% total cellular mRNA) and with control level undetectable without PCR method. Based on studies of ³⁵S methionine cell labeling, the clone has the characteristics recently discovered for the major GC-induced extracellular glycoprotein in these cells, which is a sialenated, N-glycosylated molecule with a putative inositol phosphate anchor. The induction of TIGR RNA approached 4% of the total cellular mRNA. The mRNA increased progressively over 10 days of dexamethasone treatment.

25 The II.2 clone is 2.0 Kb whereas the Northern blotting shows a band of 2.5 Kb. Although not including a poly A tail, the 3' end of the clone contains two consensus polyadenylation signals. Southern analysis suggested two groups of genomic sequences and two genomic clones were isolated. In-situ hybridization using this genomic probe showing the gene locations on chromosome 1, p36 and 12, p13, p15. Study of cyclohexamide treatment in the absence and presence of GC suggest that the induction of TIGR may involve factors in addition to the GC receptor. The TIGR gene may be involved in the cellular stress response since it is also induced by stimulants such as H₂O₂, TPA, glucose and heat; this fact may relate to glaucoma pathogenesis and treatment.

35 The amino acid sequence of TIGR, the cDNA sequence that encodes TIGR, and the 2.0 kb nucleotide sequence that contains this coding region are shown in Figures 1A-1D. The amino acid sequence of TIGR is shown in

- 6 -

Figures 1A-1D (and in SEQ ID NO:1). The nucleotide sequence of SEQ ID NO:2 is that of the TIGR cDNA molecule that is shown in Figures 1A-1D. The portion of SEQ ID NO:2 that is shown in Figures 1A-1D as encoding the TIGR protein is presented as SEQ ID NO:3.

5 The primary structure of TIGR initiates from an ATG initiation site (SEQ ID NO:2, residue 1) and includes a 20 amino acid consensus signal sequence at a second ATG (SEQ ID NO:2, residue 15), indicating that the protein is a secretory protein. The protein contains an N-linked glycosylation site located in the most hydrophilic region of the molecule. The amino
10 terminal portion of the protein is highly polarized and adopts alpha helical structure as shown by its hydropathy profile and the Garnier-Robison structure analysis. In contrast, the protein contains a 25 amino acid hydrophobic region near its carboxy terminus. This region may comprise a GIP anchoring sequence. Thus, the invention concerns two TIGR proteins:
15 TIGR and a processed form of TIGR.

 The TIGR protein also contains 5 putative O-linked glycosylation sites throughout the molecule. "Leucine zipper" regions define a helical structure that permits protein-protein binding to occur (see, generally, Tso, J.Y. *et al.*, PCT Patent Application WO93/11162; Land, K.H. *et al.*, PCT Patent
20 Application WO93/19176). The TIGR protein contain 7 leucine zipper units. The presence of the zipper regions provides a means for the TIGR molecules to bind to one another forming macromolecular and possible aggregation. Studies showing the specific binding of this molecule to HTM cells (but not to fibroblast cells) support the notion that it can influence the outflow pathway in
25 HTM tissue to cause the increased intra-ocular pressure that characterizes glaucoma and its related diseases. TIGR protein has also been successfully expressed using the baculovirus system and Sf9 insect cells. The major recombinant proteins are the two 55 kd cellular proteins encoded by the TIGR cDNA. Antibodies produced from these protein recognize both the cellular 55
30 kd proteins and the secreted 66 kd glycosylated form of these proteins in dexamethasone-treated HTM cells and in organ culture systems. *In situ* analysis of glaucomatous tissue specimens show a high expression level of this protein relative to normal controls.

 The presence, induction, and level of the TIGR secretory protein mirror
35 the onset and kinetics with which glucocorticoids induce glaucoma, and the glucocorticoid-induced expression of this secretory protein comprises the

- 7 -

molecular basis for glaucoma and its related diseases. Such an understanding of the molecular basis permits the definition of diagnostic agents for glaucoma and its related diseases.

II. The Preferred Agents of the Invention

5 As used herein, the term "glaucoma" has its art recognized meaning, and includes both primary glaucomas, secondary glaucomas, and familial (i.e. inherited glaucomas). The methods of the present invention are particularly relevant to the diagnosis of POAG, OAG, juvenile glaucoma, and inherited
10 glaucomas. A disease or condition is said to be related to glaucoma if it possesses or exhibits a symptom of glaucoma, for example, an increased intra-ocular pressure resulting from aqueous outflow resistance (see, Vaughan, D. *et al.*, In: *General Ophthalmology*, Appleton & Lange, Norwalk, CT, pp. 213-230 (1992)). The preferred agents of the present invention are discussed in detail below.

15 The agents of the present invention are capable of being used to diagnose the presence or severity of glaucoma and its related diseases in a patient suffering from glaucoma (a "glaucomatous patient"). Such agents may be either naturally occurring or non-naturally occurring. As used herein, a naturally occurring molecule may be "substantially purified," if desired, such
20 that one or more molecules that is or may be present in a naturally occurring preparation containing that molecule will have been removed or will be present at a lower concentration than that at which it would normally be found.

25 The agents of the present invention will preferably be "biologically active" with respect to either a structural attribute, such as the capacity of a nucleic acid to hybridize to another nucleic acid molecule, or the ability of a protein to be bound by antibody (or to compete with another molecule for such binding) Alternatively, such an attribute may be catalytic, and thus involve the capacity of the agent to mediate a chemical reaction or response.

30 The agents of the present invention comprise nucleic acid molecules, proteins, and organic molecules.

A. Nucleic Acid Molecules

A preferred class of agents of the present invention comprises TIGR nucleic acid molecules ("TIGR molecules"). Such molecules may be either DNA or RNA.

5 In one embodiment, such nucleic acid molecules will encode all or a fragment of TIGR protein, its "promoter" or flanking gene sequences. As used herein, the term "promoter" is used in an expansive sense to refer to the regulatory sequence(s) that control mRNA production. Such sequences include RNA polymerase binding sites, glucocorticoid response elements, enhancers, etc. All such TIGR molecules may be used to diagnose the presence of glaucoma and severity of glaucoma.

Fragment TIGR nucleic acid molecules may encode significant portion(s) of, or indeed most of, the TIGR protein. Alternatively, the fragments may comprise smaller oligonucleotides (having from about 15 to about 250 nucleotide residues, and more preferably, about 15 to about 30 nucleotide residues.). Such oligonucleotides may be used as probes of TIGR mRNA. For such purpose, the oligonucleotides must be capable of specifically hybridizing to a TIGR nucleic acid molecule. As used herein, two nucleic acid molecules are said to be capable of specifically hybridizing to one another if the two molecules are capable of forming an anti-parallel, double-stranded nucleic acid structure, whereas they are unable to form a double-stranded structure when incubated with a non-TIGR nucleic acid molecule. A nucleic acid molecule is said to be the "complement" of another nucleic acid molecule if they exhibit complete complementarity. As used herein, molecules are said to exhibit "complete complementarity" when every nucleotide of one of the molecules is complementary to a nucleotide of the other. Two molecules are said to be "minimally complementary" if they can hybridize to one another with sufficient stability to permit them to remain annealed to one another under at least conventional "low-stringency" conditions. Similarly, the molecules are said to be "complementary" if they can hybridize to one another with sufficient stability to permit them to remain annealed to one another under conventional "high-stringency" conditions. Conventional stringency conditions are described by Sambrook, J., et al., (In: *Molecular Cloning, a Laboratory Manual, 2nd Edition*, Cold Spring Harbor Press, Cold Spring Harbor, New York (1989)), and by Haymes, B.D., et al. (In: *Nucleic Acid Hybridization, A*

5 *Practical Approach*, IRL Press, Washington, DC (1985)), both herein incorporated by reference). Departures from complete complementarity are therefore permissible, as long as such departures do not completely preclude the capacity of the molecules to form a double-stranded structure. Thus, in order for an oligonucleotide to serve as a primer it need only be sufficiently complementary in sequence to be able to form a stable double-stranded structure under the particular solvent and salt concentrations employed.

10 Apart from their diagnostic uses, such oligonucleotides may be employed to obtain other TIGR nucleic acid molecules. Such molecules include the TIGR-encoding nucleic acid molecule of non-human animals (particularly, cats, monkeys, rodents and dogs), fragments thereof, as well as their promoters and flanking sequences. Such molecules can be readily obtained by using the above-described primers to screen cDNA or genomic libraries obtained from non-human species. Methods for forming such
15 libraries are well known in the art. Such analogs may differ in their nucleotide sequences from that of SEQ ID NO:1, because complete complementarity is not needed for stable hybridization. The TIGR nucleic acid molecules of the present invention therefore also include molecules that, although capable of specifically hybridizing with TIGR nucleic acid molecules may lack "complete
20 complementarity."

Any of a variety of methods may be used to obtain the above-described nucleic acid molecules. SEQ ID NO:2 may be used to synthesize all or any portion of the TIGR protein or the TIGR cDNA (Zamechik *et al.*, *Proc. Natl. Acad. Sci. (U.S.A.)* 83:4143 (1986); Goodchild *et al.*, *Proc. Natl. Acad. Sci. (U.S.A.)* 85:5507 (1988); Wickstrom *et al.*, *Proc. Natl. Acad. Sci. (U.S.A.)* 85:1028; Holt, J.T. *et al.*, *Molec. Cell. Biol.* 8:963 (1988); Gerwitz, A.M. *et al.*, *Science* 242:1303 (1988); Anfossi, G., *et al.*, *Proc. Natl. Acad. Sci. (U.S.A.)* 86:3379 (1989); Becker, D., *et al.*, *EMBO J.* 8:3679 (1989); all of which references are incorporated herein by reference). Automated nucleic acid synthesizers may be employed for this
25 purpose. In lieu of such synthesis, the disclosed SEQ ID NO:2 may be used to define a pair of primers that can be used with the polymerase chain reaction (Mullis, K. *et al.*, *Cold Spring Harbor Symp. Quant. Biol.* 51:263-273 (1986); Erlich H. *et al.*, EP 50,424; EP 84,796, EP 258,017, EP 237,362; Mullis, K., EP 201,184; Mullis K. *et al.*, US 4,683,202; Erlich, H., US 4,582,788; and Saiki, R. *et al.*, US
30 4,683,194)) to amplify and obtain any desired TIGR-encoding DNA molecule or fragment.

- 10 -

The TIGR promoter sequence(s) and TIGR flanking sequences can also be obtained using the SEQ ID NO:2 sequence provided herein. In one embodiment, such sequences are obtained by incubating oligonucleotide probes of TIGR oligonucleotides with members of genomic human libraries and recovering clones that hybridize to the probes. In a second embodiment, methods of "chromosome walking," or 3' or 5' RACE may be used (Frohman, M.A. *et al.*, *Proc. Natl. Acad. Sci. (U.S.A.)* 85:8998-9002 (1988); Ohara, O. *et al.*, *Proc. Natl. Acad. Sci. (U.S.A.)* 86:5673-5677 (1989)) to obtain such sequences.

B. TIGR Protein and Peptide Molecules

A second preferred class of agents ("TIGR molecules") comprises the TIGR protein, its peptide fragments, fusion proteins, and analogs. As used herein, the term "TIGR protein" refers to a protein having the amino acid sequence of SEQ ID NO:1. TIGR protein may be produced via chemical synthesis, or more preferably, by expressing TIGR-encoding cDNA in a suitable bacterial or eukaryotic host. Most preferably, the subsequence of such cDNA that encodes TIGR may be used for this purpose (SEQ ID NO:3). Alternatively, the entire cDNA (SEQ ID NO:2) shown in Figures 1A-1D may be employed. Suitable methods for expression are described by Sambrook, J., *et al.*, (In: *Molecular Cloning, a Laboratory Manual, 2nd Edition, Cold Spring Harbor Press, Cold Spring Harbor, New York* (1989)), or similar texts.

A "TIGR fragment" is a peptide or polypeptide whose amino acid sequence comprises a subset of the amino acid sequence of TIGR protein. A TIGR protein or fragment thereof that comprises one or more additional non-TIGR peptide regions is a "TIGR fusion" protein. Such molecules may be derivatized to contain carbohydrate or other moieties (such as keyhole limpet hemocyanin, etc.). As in the case of TIGR protein, the fragments and fusions of the present invention are preferably produced via recombinant means.

The analogs of the TIGR molecules comprise TIGR proteins, fragments or fusions in which non-essential, or not relevant, amino acid residues have been added, replaced, or deleted. An example of such an analog is the TIGR protein of non-human species, such as primates, dogs, cats, etc. Such analogs can readily be obtained by any of a variety of methods. Most preferably, as indicated above, the disclosed SEQ ID NO:2 will be used to define a pair of primers that may be used to isolate the TIGR-encoding nucleic acid molecules

- 11 -

from any desired species. Such molecules can be expressed to yield TIGR analogs by recombinant means.

C. Antibodies Reactive Against TIGR

5 One aspect of the present invention concerns antibodies, single-chain antigen binding molecules, or other proteins that specifically bind to TIGR protein and its analogs, fusions or fragments. Such antibodies are "anti-TIGR antibodies," and may be used to diagnose glaucoma and its related diseases. As used herein, an antibody or peptide is said to "specifically bind" to TIGR if
10 such binding is not competitively inhibited by the presence of non-TIGR molecules.

Nucleic acid molecules that encode all or part of the TIGR protein can be expressed, via recombinant means, to yield TIGR protein or peptides that can in turn be used to elicit antibodies that are capable of binding TIGR. Such
15 antibodies may be used in immunodiagnostic assays of glaucoma. Such TIGR-encoding molecules, or their fragments may be a "fusion" molecule (i.e. a part of a larger nucleic acid molecule) such that, upon expression, a fusion protein is produced.

The antibodies that specifically bind TIGR proteins and protein fragments may be polyclonal or monoclonal, and may comprise intact
20 immunoglobulins, of antigen binding portions of immunoglobulins (such as (F(ab'), F(ab')₂) fragments, or single-chain immunoglobulins producible, for example, via recombinant means.

Murine monoclonal antibodies are particularly preferred. BALB/c mice are preferred for this purpose, however, equivalent strains may also be
25 used. The animals are preferably immunized with approximately 25 µg of purified TIGR protein (or fragment thereof) that has been emulsified a suitable adjuvant (such as TiterMax adjuvant (Vaxcel, Norcross, GA)). Immunization is preferably conducted at two intramuscular sites, one intraperitoneal site, and one subcutaneous site at the base of the tail. An additional i.v. injection of
30 approximately 25 µg of antigen is preferably given in normal saline three weeks later. After approximately 11 days following the second injection, the mice may be bled and the blood screened for the presence of anti-TIGR antibodies. Preferably, a direct binding ELISA is employed for this purpose.

Most preferably, the mouse having the highest antibody titer is given a
35 third i.v. injection of approximately 25 µg of TIGR protein or fragment. The

- 12 -

splenic leukocytes from this animal may be recovered 3 days later, and are then permitted to fuse, most preferably, using polyethylene glycol, with cells of a suitable myeloma cell line (such as, for example, the P3X63Ag8.653 myeloma cell line). Hybridoma cells are selected by culturing the cells under
5 "HAT" (hypoxanthine-aminopterin-thymine) selection for about one week. The resulting clones may then be screened for their capacity to produce monoclonal antibodies ("mAbs) to TIGR protein, preferably by direct ELISA.

In one embodiment, anti-TIGR monoclonal antibodies are isolated using TIGR fusions, or conjugates, as immunogens. Thus, for example, a
10 group of mice can be immunized using a TIGR fusion protein emulsified in Freund's complete adjuvant (approximately 50 µg of antigen per immunization). At three week intervals, an identical amount of antigen is emulsified in Freund's incomplete adjuvant and used to immunize the animals. Ten days following the third immunization, serum samples are taken
15 and evaluated for the presence of antibody. If antibody titers are too low, a fourth booster can be employed. Polysera capable of binding TIGR at 1:5,000 dilution can also be obtained using this method.

In a preferred procedure for obtaining monoclonal antibodies, the spleens of the above-described immunized mice are removed, disrupted, and
20 immune splenocytes are isolated over a ficoll gradient. The isolated splenocytes are fused, using polyethylene glycol with BALB/c-derived HGPRT (hypoxanthine guanine phosphoribosyl transferase) deficient P3x63xAg8.653 plasmacytoma cells. The fused cells are plated into 96 well microtiter plates and screened for hybridoma fusion cells by their capacity to
25 grow in culture medium supplemented with hypoxanthine, aminopterin and thymidine for approximately 2-3 weeks. On average, out of every 10^6 spleen cells subjected to fusion yields a viable hybridoma. A typical spleen yields $5-10 \times 10^7$ spleen cells.

Hybridoma cells that arise from such incubation are preferably
30 screened for their capacity to produce an immunoglobulin that binds to TIGR protein. An indirect ELISA may be used for this purpose. In brief, the supernatants of hybridomas are incubated in microtiter wells that contain immobilized TIGR protein. After washing, the titer of bound immunoglobulin can be determined using, for example, a goat anti-mouse antibody conjugated
35 to horseradish peroxidase. After additional washing, the amount of immobilized enzyme is determined (for example through the use of a

- 13 -

chromogenic substrate). Such screening is performed as quickly as possible after the identification of the hybridoma in order to ensure that a desired clone is not overgrown by non-secreting neighbors. Desirably, the fusion plates are screened several times since the rates of hybridoma growth vary. In a preferred sub-embodiment, a different antigenic form of TIGR may be used to screen the hybridoma. Thus, for example, the splenocytes may be immunized with one TIGR immunogen, but the resulting hybridomas can be screened using a different TIGR immunogen.

As discussed below, such antibody molecules or their fragments may be used for diagnostic purposes. Where the antibodies are intended for diagnostic purposes, it may be desirable to derivatize them, for example with a ligand group (such as biotin) or a detectable marker group (such as fluorescent group, a radioisotope or an enzyme).

The ability to produce antibodies that bind TIGR molecules permits the identification of mimetic compounds of TIGR. A "mimetic compound" of TIGR is a compound that is not TIGR, or a fragment of TIGR, but which nonetheless exhibits an ability to specifically bind to anti-TIGR antibodies. Such molecules can be used to elicit anti-TIGR antibodies, and thus, may be used to assist the diagnosis of glaucoma and its related diseases.

III. Uses of the Molecules of the Invention in the Diagnosis of Glaucoma and Related Diseases

A particularly desired use of the present invention relates to the diagnosis of glaucoma, POAG, pigmentary glaucoma, high tension glaucoma and low tension glaucoma and their related diseases. As used herein the term "glaucoma" refers to glaucoma, POAG, pigmentary glaucoma, and low tension glaucoma and their related diseases. As indicated above, methods for diagnosing glaucoma suffer from inaccuracy, or require multiple examinations. The molecules of the present invention may be used to define superior assays for glaucoma. Quite apart from such usage, the molecules of the present invention may be used to diagnosis or predict an individual's sensitivity to elevated intraocular pressure upon administration of steroids such as glucocorticoids or corticosteroids). Dexamethasone, cortisol and prednisolone are preferred steroids for this purpose. Medical conditions such as inflammatory and allergic disorders, as well as organ transplantation

recipients, benefit from treatment with glucocorticoids. Certain individuals exhibit an increased sensitivity to such steroids (i.e., "steroid sensitivity"), which is manifested by an undesired increase in intraocular pressure. The present invention may be employed to diagnosis or predict such sensitivity, as well as glaucoma and related diseases.

In a first embodiment, the TIGR molecules of the present invention are used to determine whether an individual has a mutation affecting the level (i.e., the concentration of TIGR mRNA or protein in a sample, etc.) or pattern (i.e., the kinetics of expression, rate of decomposition, stability profile, etc.) of the TIGR expression (collectively, the "TIGR Response" of a cell or bodily fluid) (for example, a mutation in the TIGR gene, or in a regulatory region(s) or other gene(s) that control or affect the expression of TIGR), and being predictive of individuals who would be predisposed to glaucoma, related diseases, or steroid sensitivity. As used herein, the TIGR Response manifested by a cell or bodily fluid is said to be "altered" if it differs from the TIGR Response of cells or of bodily fluids of normal individuals. Such alteration may be manifested by either abnormally increased or abnormally diminished TIGR Response. To determine whether a TIGR Response is altered, the TIGR Response manifested by the cell or bodily fluid of the patient is compared with that of a similar cell sample (or bodily fluid sample) of normal individuals. As will be appreciated, it is not necessary to re-determine the TIGR Response of the cell sample (or bodily fluid sample) of normal individuals each time such a comparison is made; rather, the TIGR Response of a particular individual may be compared with previously obtained values of normal individuals.

In one sub-embodiment, such an analysis is conducted by determining the presence and/or identity of polymorphism(s) in the TIGR gene or its flanking regions which are associated with glaucoma, or a predisposition to glaucoma, related diseases, or steroid sensitivity.

Any of a variety of molecules can be used to identify such polymorphism(s). In one embodiment, the TIGR cDNA sequence (or a sub-sequence thereof) may be employed as a marker nucleic acid molecule to identify such polymorphism(s). Alternatively, such polymorphisms can be detected through the use of a marker nucleic acid molecule or a marker protein that is genetically linked to (i.e., a polynucleotide that co-segregates with) such polymorphism(s). As stated above, the TIGR gene has been

- 15 -

mapped to p36 of chromosome 1, and to p13, q15 of chromosome 10, 11, or 12. In a preferred aspect of this embodiment, such marker nucleic acid molecules will have the nucleotide sequence of a polynucleotide that is closely genetically linked to such polymorphism(s) (e.g., markers located at
5 chromosome 1, p and chromosome 12, p, and more preferably to chromosome 1, p34-p38 and chromosome 12, p11-p17. Polynucleotide markers that map to such locations are well known and can be employed to identify such polymorphism(s).

10 In an alternative embodiment, such polymorphisms can be detected through the use of a marker nucleic acid molecule that is physically linked to such polymorphism(s). For this purpose, marker nucleic acid molecules comprising a nucleotide sequence of a polynucleotide located within 1 mb of the polymorphism(s), and more preferably within 100 kb of the polymorphism(s), and most preferably within 10 kb of the polymorphism(s)
15 can be employed.

The genomes of animals and plants naturally undergo spontaneous mutation in the course of their continuing evolution (Gusella, J.F., *Ann. Rev. Biochem.* 55:831-854 (1986)).

20 A "polymorphism" in the TIGR gene or its flanking regions is a variation or difference in the sequence of the TIGR gene or its flanking regions that arises in some of the members of a species. The variant sequence and the "original" sequence co-exist in the species' population. In some instances, such co-existence is in stable or quasi-stable equilibrium.

25 A polymorphism is thus said to be "allelic," in that, due to the existence of the polymorphism, some members of a species may have the original sequence (i.e. the original "allele") whereas other members may have the variant sequence (i.e. the variant "allele"). In the simplest case, only one variant sequence may exist, and the polymorphism is thus said to be di-allelic. In other cases, the species' population may contain multiple alleles, and the
30 polymorphism is termed tri-allelic, etc. A single gene may have multiple different unrelated polymorphisms. For example, it may have a di-allelic polymorphism at one site, and a multi-allelic polymorphism at another site.

35 The variation that defines the polymorphism may range from a single nucleotide variation to the insertion or deletion of extended regions within a gene. In some cases, the DNA sequence variations are in regions of the genome that are characterized by short tandem repeats (STRs) that include

- 16 -

tandem di- or tri-nucleotide repeated motifs of nucleotides. Polymorphisms characterized by such tandem repeats are referred to as "variable number tandem repeat" ("VNTR") polymorphisms. VNTRs have been used in identity and paternity analysis (Weber, J.L., U.S. Patent 5,075,217; Armour, J.A.L. *et al.*, *FEBS Lett.* 307:113-115 (1992); Jones, L. *et al.*, *Eur. J. Haematol.* 39:144-147 (1987); Horn, G.T. *et al.*, PCT Application WO91/14003; Jeffreys, A.J., European Patent Application 370,719; Jeffreys, A.J., U.S. Patent 5,175,082); Jeffreys, A.J. *et al.*, *Amer. J. Hum. Genet.* 39:11-24 (1986); Jeffreys, A.J. *et al.*, *Nature* 316:76-79 (1985); Gray, I.C. *et al.*, *Proc. R. Acad. Soc. Lond.* 243:241-253 (1991); Moore, S.S. *et al.*, *Genomics* 10:654-660 (1991); Jeffreys, A.J. *et al.*, *Anim. Genet.* 18:1-15 (1987); Hillel, J. *et al.*, *Anim. Genet.* 20:145-155 (1989); Hillel, J. *et al.*, *Genet.* 124:783-789 (1990)).

The detection of polymorphic sites in a sample of DNA may be facilitated through the use of nucleic acid amplification methods. Such methods specifically increase the concentration of polynucleotides that span the polymorphic site, or include that site and sequences located either distal or proximal to it. Such amplified molecules can be readily detected by gel electrophoresis or other means.

The most preferred method of achieving such amplification employs the polymerase chain reaction ("PCR") (Mullis, K. *et al.*, *Cold Spring Harbor Symp. Quant. Biol.* 51:263-273 (1986); Erlich H. *et al.*, European Patent Appln. 50,424; European Patent Appln. 84,796, European Patent Application 258,017, European Patent Appln. 237,362; Mullis, K., European Patent Appln. 201,184; Mullis K. *et al.*, U.S. Patent No. 4,683,202; Erlich, H., U.S. Patent No. 4,582,788; and Saiki, R. *et al.*, U.S. Patent No. 4,683,194), using primer pairs that are capable of hybridizing to the proximal sequences that define a polymorphism in its double-stranded form.

In lieu of PCR, alternative methods, such as the "Ligase Chain Reaction" ("LCR") may be used (Barany, F., *Proc. Natl. Acad. Sci. (U.S.A.)* 88:189-193 (1991). LCR uses two pairs of oligonucleotide probes to exponentially amplify a specific target. The sequences of each pair of oligonucleotides is selected to permit the pair to hybridize to abutting sequences of the same strand of the target. Such hybridization forms a substrate for a template-dependent ligase. As with PCR, the resulting products thus serve as a template in subsequent cycles and an exponential amplification of the desired sequence is obtained.

- 17 -

LCR can be performed with oligonucleotides having the proximal and distal sequences of the same strand of a polymorphic site. In one embodiment, either oligonucleotide will be designed to include the actual polymorphic site of the polymorphism. In such an embodiment, the reaction conditions are selected such that the oligonucleotides can be ligated together only if the target molecule either contains or lacks the specific nucleotide that is complementary to the polymorphic site present on the oligonucleotide. Alternatively, the oligonucleotides may be selected such that they do not include the polymorphic site (see, Segev, D., PCT Application WO 90/01069).

The "Oligonucleotide Ligation Assay" ("OLA") may alternatively be employed (Landegren, U. *et al.*, *Science* 241:1077-1080 (1988)). The OLA protocol uses two oligonucleotides which are designed to be capable of hybridizing to abutting sequences of a single strand of a target. OLA, like LCR, is particularly suited for the detection of point mutations. Unlike LCR, however, OLA results in "linear" rather than exponential amplification of the target sequence.

Nickerson, D.A. *et al.* have described a nucleic acid detection assay that combines attributes of PCR and OLA (Nickerson, D.A. *et al.*, *Proc. Natl. Acad. Sci. (U.S.A.)* 87:8923-8927 (1990)). In this method, PCR is used to achieve the exponential amplification of target DNA, which is then detected using OLA. In addition to requiring multiple, and separate, processing steps, one problem associated with such combinations is that they inherit all of the problems associated with PCR and OLA.

Schemes based on ligation of two (or more) oligonucleotides in the presence of nucleic acid having the sequence of the resulting "di-oligonucleotide", thereby amplifying the di-oligonucleotide, are also known (Wu, D.Y. *et al.*, *Genomics* 4:560 (1989)), and may be readily adapted to the purposes of the present invention.

Other known nucleic acid amplification procedures, such as allele-specific oligomers, branched DNA technology, transcription-based amplification systems, or isothermal amplification methods may also be used to amplify and analyze such polymorphisms (Malek, L.T. *et al.*, U.S. Patent 5,130,238; Davey, C. *et al.*, European Patent Application 329,822; Schuster *et al.*, U.S. Patent 5,169,766; Miller, H.I. *et al.*, PCT appln. WO 89/06700; Kwoh, D. *et al.*, *Proc. Natl. Acad. Sci. (U.S.A.)* 86:1173 (1989); Gingeras, T.R. *et al.*, PCT application WO 88/10315; Walker, G.T. *et al.*, *Proc. Natl. Acad. Sci.*

(U.S.A.) 89:392-396 (1992)). All the foregoing nucleic acid amplification methods could be used to predict or diagnose glaucoma.

5 The identification of a polymorphism in the TIGR gene can be determined in a variety of ways. By correlating the presence or absence of
glaucoma in an individual with the presence or absence of a polymorphism in
10 the TIGR gene or its flanking regions, it is possible to diagnose the predisposition of an asymptomatic patient to glaucoma, related diseases, or steroid sensitivity. If a polymorphism creates or destroys a restriction endonuclease cleavage site, or if it results in the loss or insertion of DNA (e.g.,
a VNTR polymorphism), it will alter the size or profile of the DNA fragments that are generated by digestion with that restriction endonuclease. As such, individuals that possess a variant sequence can be distinguished from those
15 having the original sequence by restriction fragment analysis. Polymorphisms that can be identified in this manner are termed "restriction fragment length polymorphisms" ("RFLPs"). RFLPs have been widely used in human and animal genetic analyses (Glassberg, J., UK patent Application 2135774; Skolnick, M.H. *et al.*, *Cytogen. Cell Genet.* 32:58-67 (1982); Botstein, D. *et al.*, *Ann. J. Hum. Genet.* 32:314-331 (1980); Fischer, S.G *et al.* (PCT Application WO90/13668); Uhlen, M., PCT Application WO90/11369)). The role of TIGR
20 in glaucoma pathogenesis indicates that the presence of genetic alterations (e.g., DNA polymorphisms) that affect the TIGR Response can be employed to predict glaucoma .

In accordance with this embodiment of the invention, a sample DNA is obtained from a patient's cells. In a preferred embodiment, the DNA sample
25 is obtained from the patient's blood. However, any source of DNA may be used. The DNA is subjected to restriction endonuclease digestion. TIGR is used as a probe in accordance with the above-described RFLP methods. By comparing the RFLP pattern of the TIGR gene obtained from normal and glaucomatous patients, one can determine a patient's predisposition to
30 glaucoma. The polymorphism obtained in this approach can then be cloned to identify the mutation at the coding region which alters the protein's structure or regulatory region of the gene which affects its expression level. Changes involving promoter interactions with other regulatory proteins can be identified by, for example, gel shift assays using HTM cell extracts, fluid from
35 the anterior chamber of the eye, serum, etc. Interactions of TIGR protein in glaucomatous cell extracts, fluid from the anterior chamber of the eye, serum,

- 19 -

etc. can be compared to control samples to thereby identify changes in those properties of TIGR that relate to the pathogenesis of glaucoma. Similarly such extracts and fluids as well as others (blood, etc.) can be used to diagnosis or predict steroid sensitivity.

5 Several different classes of polymorphisms may be identified through such methods. Examples of such classes include: (1) polymorphisms present in the TIGR cDNA of different individuals; (2) polymorphisms in non-translated TIGR gene sequences, including the promoter or other regulatory regions of the TIGR gene; (3) polymorphisms in genes whose products interact
10 with TIGR regulatory sequences; (4) polymorphisms in gene sequences whose products interact with the TIGR protein, or to which the TIGR protein binds.

 In an alternate sub-embodiment, the evaluation is conducted using oligonucleotide "probes" whose sequence is complementary to that of a portion of TIGR mRNA. Such molecules are then incubated with cell extracts
15 of a patient under conditions sufficient to permit nucleic acid hybridization. For this sub-embodiment, cells of the trabecular meshworks are preferred. The detection of double-stranded probe-mRNA hybrid molecules is indicative of the presence of TIGR mRNA; the amount of such hybrid formed is proportional to the amount of TIGR mRNA. Thus, such probes may be used
20 to ascertain the level and extent of TIGR mRNA production in a patient's cells. Such nucleic acid hybridization may be conducted under quantitative conditions (thereby providing a numerical value of the amount of TIGR mRNA present). Alternatively, the assay may be conducted as a qualitative assay that indicates either that TIGR mRNA is present, or that its level exceeds
25 a user set, predefined value.

 In a second embodiment, the previously described "anti-TIGR antibodies" are employed in an immunodiagnostic assay for glaucoma and its related diseases.

 As discussed above, TIGR protein is secreted extracellularly from the
30 trabecular meshwork into the extracellular matrix of the trabecular meshwork, and thus may pass out into the body fluids. This characteristic permits one to assay TIGR concentrations in blood, lymph, or serum, and to thereby determine whether a patient's TIGR levels exceed those found in the blood of individuals who do not have glaucoma and are not predisposed to glaucoma
35 related diseases or steroid sensitivity. Patients found to have altered levels of TIGR thus may be diagnosed as glaucoma.

- 20 -

Thus, a third aspect of the present invention concerns the recognition that one can diagnosis or predict glaucoma, related diseases or steroid sensitivity by determining the TIGR Response of cells or tissue other than the trabecular meshwork.

5 In one sub-embodiment of this aspect of the present invention, one can diagnose or predict glaucoma, related diseases and steroid sensitivity by ascertaining the TIGR Response in a biopsy (or a macrophage or other blood cell sample), or other cell sample, or more preferably, in a sample of bodily fluid (especially, blood, serum, plasma, tears, etc.). Since the TIGR gene is
10 induced in response to the presence of glucocorticoids, a highly preferred embodiment of this method comprises ascertaining such TIGR Response prior to, during and/or subsequent to, the administration of a glucocorticoid. Thus, by way of illustration, glaucoma could be diagnosed or predicted by determining whether the administration of a glucocorticoid (administered
15 topically, intraocularly, intramuscularly, systemically, or otherwise) alters the TIGR Response of a particular individual, relative to that of normal individuals. Most preferably, for this purpose, at least a "TIGR gene-inducing amount" of the glucocorticoid will be provided. As used herein, a TIGR gene-inducing amount of a glucocorticoid is an amount of glucocorticoid sufficient
20 to cause a detectable induction of TIGR expression in cells of glaucomatous or non-glaucomatous individuals.

The anti-TIGR antibodies of the present invention may thus be used in an immunoassay to assess the presence of TIGR. Any of a wide array of immunoassays formats may be used for this purpose (Fackrell, *J. Clin. Immunoassay* 8:213-219 (1985)), Yolken, R.H., *Rev. Infect. Dis.* 4:35 (1982);
25 Collins, W.P., In: *Alternative Immunoassays*, John Wiley & Sons, NY (1985); Ngo, T.T. et al., In: *Enzyme Mediated Immunoassay*, Plenum Press, NY (1985)).

The simplest immunoassay involves merely incubating an antibody that is capable of binding to a predetermined target molecule with a sample suspected to contain the target molecule. The presence of the target molecule
30 is determined by the presence, and proportional to the concentration, of any antibody bound to the target molecule. In order to facilitate the separation of target-bound antibody from the unbound antibody initially present, a solid phase is typically employed. Thus, for example the sample can be passively
35 bound to a solid support, and, after incubation with the antibody, the support can be washed to remove any unbound antibody.

- 21 -

5 In more sophisticated immunoassays, the concentration of the target molecule is determined by binding the antibody to a support, and then permitting the support to be in contact with a sample suspected of containing the target molecule. Target molecules that have become bound to the immobilized antibody can be detected in any of a variety of ways. For example, the support can be incubated in the presence of a labeled, second antibody that is capable of binding to a second epitope of the target molecule. Immobilization of the labeled antibody on the support thus requires the presence of the target, and is proportional to the concentration of the target in the sample. In an alternative assay, the target is incubated with the sample and with a known amount of labeled target. The presence of target molecule in the sample competes with the labeled target molecules for antibody binding sites. Thus, the amount of labeled target molecules that are able to bind the antibody is inversely proportional to the concentration of target molecule in the sample.

15 In general, immunoassay formats employ either radioactive labels ("RIAs") or enzyme labels ("ELISAs"). RIAs have the advantages of simplicity, sensitivity, and ease of use. Radioactive labels are of relatively small atomic dimension, and do not normally affect reaction kinetics. Such assays suffer, however, from the disadvantages that, due to radioisotopic decay, the reagents have a short shelf-life, require special handling and disposal, and entail the use of complex and expensive analytical equipment. RIAs are described in *Laboratory Techniques and Biochemistry in Molecular Biology*, by Work, T.S., et al., North Holland Publishing Company, NY (1978), with particular reference to the chapter entitled "An Introduction to Radioimmune Assay and Related Techniques" by Chard, T., incorporated by reference herein. ELISAs have the advantage that they can be conducted using inexpensive equipment, and with a myriad of different enzymes, such that a large number of detection strategies --colorimetric, pH, gas evolution, etc. -- can be used to quantitate the assay. In addition, the enzyme reagents have relatively long shelf-lives, and lack the risk of radiation contamination that attends to RIA use. ELISAs are described in *ELISA and Other Solid Phase Immunoassays* (Kemeny, D.M. et al., Eds.), John Wiley & Sons, NY (1988), incorporated by reference herein.

- 22 -

In an alternative diagnostic format, ocular tissue (obtained, for example by trabeculotomy) may be evaluated in an *in situ* immunodiagnostic assay for glaucoma and its related diseases.

5 In such a format, antibodies (especially labeled antibodies) or other TIGR-binding peptides are incubated in the presence of ocular tissue in order to evaluate the clinical degree and significance of glaucoma in biopsied tissue. The extent, location, or degree of TIGR in the ocular tissue is determined by staining or other visualization methods. Such information is then compared to the staining pattern obtained from normal or glaucomatous individuals in
10 order to diagnose or predict glaucoma.

Anti-TIGR antibodies or TIGR binding molecules may be administered to a patient, and their capacity to bind to TIGR *in vivo* may be determined by ocular examination. Significantly, since such a diagnostic test is relatively rapid, immune responses that require significant time, such as the potential
15 eliciting of anti-[anti-TIGR] antibodies, or the complexing of such antibodies with anti-TIGR antibodies is of not important. In a preferred embodiment, the antibody will be fluorescently labeled, and will be provided to a patient by injection into the patient's circulatory system. The antibody progresses from the circulatory system to the posterior optic chamber. The complexing of the
20 antibody with TIGR can be monitored using conventional gonioscopy, or by other suitable means. Significantly, such an assay provides both a means to visualize the trabecular meshwork and a means for determining the extent of deposited TIGR in the extracellular matrix.

As discussed above, TIGR protein exhibits an ability to self-aggregate,
25 due at least in part to the presence of leucine zippers in the molecule. Because small peptide fragments of TIGR that possess such zipper regions can bind to TIGR, such peptides may be used as alternatives to anti-TIGR antibodies in diagnostic assays. The use of such peptides is desirable since the peptides can be modified to possess both lipophilic and hydrophilic groups. The presence
30 of such groups will permit the peptide to traverse the corneal membrane. Thus, such agents may be provided topically in an eye drop or ointment, and can be used in the same manner as anti-TIGR antibodies to effect the diagnosis of glaucoma. The peptide will desirably be labeled with a fluorescent group to facilitate detection.

35 Any suitable peptide fragment of TIGR may be used for this purpose, however, it is preferable to use a fragment corresponding to all or part of SEQ

ID NO:1 residues 85-92, 92-99, 121-128; 128-135, 135-142, 142-149, 149-156, 241-248 and 374-381. Suitable lipophilic and hydrophilic groups are known in the art (see, Remington's Pharmaceutical Sciences), and comprise aliphatic groups, lipids, etc. (lipophilic groups) and organic acids, esters, ionic groups, etc. (hydrophilic groups). Such groups can be readily added to the TIGR molecules of the present invention by, for example, derivatizing the side chain groups of appropriate amino acids.

Cysteiny l residues may be reacted with α -haloacetates (and corresponding amines), such as chloroacetic acid or chloroacetamide, to give carboxymethyl or carboxyamidomethyl derivatives. Cysteiny l residues also are derivatized by reaction with bromotrifluoroacetone, α -bromo-b-(5-imidazolyl)propionic acid, chloroacetyl phosphate, N-alkylmaleimides, 3-nitro-2-pyridyl disulfide, methyl 2-pyridyl disulfide, p-chloromercuribenzoate, 2-chloromercuri-4-nitrophenol, or chloro-7-nitrobenzo-2-oxa-1,3-diazole.

Histidyl residues may be derivatized by reaction with diethylprocarbamate at pH 5.5-7.0 because this agent is relatively specific for the histidyl side chain. Para-bromophenacyl bromide also is useful; the reaction is preferably performed in 0.1 M sodium cacodylate at pH 6.0.

Lysiny l and amino terminal residues may be reacted with succinic or other carboxylic acid anhydrides. Derivatization with these agents has the effect of reversing the charge of the lysiny l residues. Other suitable reagents for derivatizing α -amino-containing residues include imidoesters such as methyl picolinimide; pyridoxal phosphate; pyridoxal; chloroborohydride; trinitrobenzenesulfonic acid; O-methylisourea; 2,4 pentanedione; and transaminase-catalyzed reaction with glyoxylate.

Arginy l residues may be modified by reaction with one or several conventional reagents, among them phenylglyoxal, 2,3-butanedione, 1,2-cyclohexanedione, and ninhydrin. Derivatization of arginine residues requires that the reaction be performed in alkaline conditions because of the high pK_a of the guanidine functional group. Furthermore, these reagents may react with the groups of lysine as well as the arginine epsilon-amino group.

Carboxyl side groups (aspartyl or glutamyl) may be selectively modified by reaction with carbodiimides ($R'-N-C-N-R'$) such as 1-cyclohexyl-3-(2-morpholinyl-(4-ethyl) carbodiimide or 1-ethyl-3 (4 azonia 4,4-dimethylpentyl) carbodiimide. Furthermore, aspartyl and glutamyl residues may be

- 24 -

converted to asparaginy and glutaminy residues by reaction with ammonium ions.

IV. Methods of Administration

5 The agents of the present invention can be formulated according to known methods to prepare pharmacologically acceptable compositions, whereby these materials, or their functional derivatives, having the desired degree of purity are combined in admixture with a physiologically acceptable carrier, excipient, or stabilizer. Such materials are non-toxic to recipients at the
10 dosages and concentrations employed. The active component of such compositions may be TIGR protein, TIGR fusion proteins or fragments of TIGR protein or analogs or mimetics of such molecules. Where nucleic acid molecules are employed, such molecules may be sense, antisense or triplex oligonucleotides of the TIGR cDNA or gene.

15 A composition is said to be "pharmacologically acceptable" if its administration can be tolerated by a recipient patient. An agent is physiologically significant if its presence results in a detectable change in the physiology of a recipient patient.

20 Suitable vehicles and their formulation, inclusive of other human proteins, e.g., human serum albumin, are described, for example, in Remington's Pharmaceutical Sciences (16th ed., Osol, A., Ed., Mack, Easton PA (1980)).

25 If the composition is to be water soluble, it may be formulated in a buffer such as phosphate or other organic acid salt preferably at a pH of about 7 to 8. If the composition is only partially soluble in water, it may be prepared as a microemulsion by formulating it with a nonionic surfactant such as Tween, Pluronic, or PEG, e.g., Tween 80, in an amount of, for example, 0.04-0.05% (w/v), to increase its solubility. The term "water soluble" as applied to the polysaccharides and polyethylene glycols is meant to include colloidal
30 solutions and dispersions. In general, the solubility of the cellulose derivatives is determined by the degree of substitution of ether groups, and the stabilizing derivatives useful herein should have a sufficient quantity of such ether groups per anhydroglucose unit in the cellulose chain to render the derivatives water soluble. A degree of ether substitution of at least 0.35 ether

- 25 -

groups per anhydroglucose unit is generally sufficient. Additionally, the cellulose derivatives may be in the form of alkali metal salts, for example, the Li, Na, K or Cs salts.

5 Optionally other ingredients may be added such as antioxidants, e.g., ascorbic acid; low molecular weight (less than about ten residues) polypeptides, e.g., polyarginine or tripeptides; proteins, such as serum albumin, gelatin, or immunoglobulins; hydrophilic polymers such as polyvinyl pyrrolidone; amino acids, such as glycine, glutamic acid, aspartic acid, or arginine; monosaccharides, disaccharides, and other carbohydrates
10 including cellulose or its derivatives, glucose, mannose, or dextrans; chelating agents such as EDTA; and sugar alcohols such as mannitol or sorbitol.

 Additional pharmaceutical methods may be employed to control the duration of action. Controlled or sustained release preparations may be achieved through the use of polymers to complex or absorb the TIGR
15 molecule(s) of the composition. The controlled delivery may be exercised by selecting appropriate macromolecules (for example polyesters, polyamino acids, polyvinyl pyrrolidone, ethylenevinylacetate, methylcellulose, carboxymethylcellulose, or protamine sulfate) and the concentration of macromolecules as well as the methods of incorporation in order to control
20 release.

 Sustained release formulations may also be prepared, and include the formation of microcapsular particles and implantable articles. For preparing sustained-release compositions, the TIGR molecule(s) of the composition is preferably incorporated into a biodegradable matrix or microcapsule. A
25 suitable material for this purpose is a polylactide, although other polymers of poly-(α -hydroxycarboxylic acids), such as poly-D-(-)-3-hydroxybutyric acid (EP 133,988A), can be used. Other biodegradable polymers include poly(lactones), poly(orthoesters), polyamino acids, hydrogels, or poly(orthocarbonates) poly(acetals). The polymeric material may also
30 comprise polyesters, poly(lactic acid) or ethylene vinylacetate copolymers. For examples of sustained release compositions, see U.S. Patent No. 3,773,919, EP 58,481A, U.S. Patent No. 3,887,699, EP 158,277A, Canadian Patent No. 1176565, Sidman, U. *et al.*, *Biopolymers* 22:547 (1983), and Langer, R. *et al.*, *Chem. Tech.* 12:98 (1982).

35 Alternatively, instead of incorporating the TIGR molecule(s) of the composition into polymeric particles, it is possible to entrap these materials in

microcapsules prepared, for example, by coacervation techniques or by interfacial polymerization, for example, hydroxymethylcellulose or gelatine-microcapsules and poly(methylmethacrylate) microcapsules, respectively, or in colloidal drug delivery systems, for example, liposomes, albumin microspheres, microemulsions, nanoparticles, and nanocapsules or in macroemulsions. Such techniques are disclosed in Remington's Pharmaceutical Sciences (1980).

In an alternative embodiment, liposome formulations and methods that permit intracellular uptake of the molecule will be employed. Suitable methods are known in the art, see, for example, Chicz, R.M. *et al.* (PCT Application WO 94/04557), Jaysena, S.D. *et al.* (PCT Application WO93/12234), Yarosh, D.B. (U.S. Patent No. 5,190,762), Callahan, M.V. *et al.* (U.S. Patent No. 5,270,052) and Gonzalezro, R.J. (PCT Application 91/05771), all herein incorporated by reference.

The pharmaceutical compositions of the present invention may be sterilized, as by filtration through sterile filtration membranes (e.g., 0.2 micron membranes). The compositions may be stored in lyophilized form or as a liquid solution. It will be understood that use of certain of the foregoing excipients, carriers, or stabilizers will result in the formation of salts of the molecules.

The compositions of the present invention can be applied topically as to the skin, or to the cornea. When applied topically, the molecule(s) of the composition may be suitably combined with other ingredients, such as carriers and/or adjuvants. There are no limitations on the nature of such other ingredients, except that they must be pharmaceutically acceptable and efficacious for their intended administration, and cannot degrade the activity of the active ingredients of the composition. Examples of suitable vehicles include ointments, creams, gels, or suspensions, with or without purified collagen. The compositions also may be impregnated into transdermal patches, and bandages, preferably in liquid or semi-liquid form.

For obtaining a gel formulation, the molecule(s) of the composition formulated in a liquid composition may be mixed with an effective amount of a water-soluble polysaccharide or synthetic polymer such as polyethylene glycol to form a gel of the proper viscosity to be applied topically. The polysaccharide that may be used includes, for example, cellulose derivatives such as etherified cellulose derivatives, including alkyl celluloses,

- 27 -

hydroxyalkyl celluloses, and alkylhydroxyalkyl celluloses, for example, methylcellulose, hydroxyethyl cellulose, carboxymethyl cellulose, hydroxypropyl methylcellulose, and hydroxypropyl cellulose; starch and fractionated starch; agar; alginic acid and alginates; gum arabic; pullullan; 5 agarose; carrageenan; dextrans; dextrans; fructans; inulin; mannans; xylans; arabinans; chitosans; glycogens; glucans; and synthetic biopolymers; as well as gums such as xanthan gum; guar gum; locust bean gum; gum arabic; tragacanth gum; and karaya gum; and derivatives and mixtures thereof. The preferred gelling agent herein is one that is inert to biological systems, non- 10 toxic, simple to prepare, and not too runny or viscous, and will not destabilize the TIGR molecule(s) held within it. Preferably the polysaccharide is an etherified cellulose derivative, more preferably one that is well defined, purified, and listed in USP, e.g., methylcellulose and the hydroxyalkyl cellulose derivatives, such as hydroxypropyl cellulose, hydroxyethyl cellulose, 15 and hydroxypropyl methylcellulose. Most preferred herein is methylcellulose.

The polyethylene glycol useful for gelling is typically a mixture of low and high molecular weight polyethylene glycols to obtain the proper viscosity. For example, a mixture of a polyethylene glycol of molecular weight 400-600 with one of molecular weight 1500 would be effective for this purpose when 20 mixed in the proper ratio to obtain a paste.

The compositions of the present invention can also be formulated for administration parenterally by injection, rapid infusion, nasopharyngeal absorption (intranasopharyngeally), dermoabsorption, or orally. The compositions may alternatively be administered intramuscularly, or 25 intravenously. Compositions for parenteral administration include sterile aqueous or non-aqueous solutions, suspensions, and emulsions. Examples of non-aqueous solvents are propylene glycol, polyethylene glycol, vegetable oils such as olive oil, and injectable organic esters such as ethyl oleate. Carriers, adjuncts or occlusive dressings can be used to increase tissue permeability and enhance antigen absorption. Liquid dosage forms for oral administration may 30 generally comprise a liposome solution containing the liquid dosage form. Suitable forms for suspending liposomes include emulsions, suspensions, solutions, syrups, and elixirs containing inert diluents commonly used in the art, such as purified water. Besides the inert diluents, such compositions can 35 also include wetting agents, emulsifying and suspending agents, or sweetening, flavoring, coloring or perfuming agents.

If methylcellulose is employed in the gel, preferably it comprises about 2-5%, more preferably about 3%, of the gel and the TIGR molecule(s) of the composition is present in an amount of about 300-1000 μg per ml of gel. The dosage to be employed is dependent upon the factors described above. As a
5 general proposition, the TIGR molecule(s) of the composition is formulated and delivered to the target site or tissue at a dosage capable of establishing in the tissue a maximum dose that is efficacious but not unduly toxic.

In the most preferred embodiment, the molecules of the invention will be provided to the cornea or surface of the eye, and permitted to adsorb across
10 the cornea into the anterior chamber of the eye. Methods that may be used for accomplishing such ocular drug delivery are described by Zun, L.S. (*Emerg. Med. Clin. North. Amer.* 6:121 (1988)), Lee, V.H. (*J. Ocular Pharmacol.* 6:157 (1990)), Ellis, P.P. (In: *Ocular Therapeutics and Pharmacology*, 7th ed., Mosby, (1987)) and (Vaughan, D. *et al.*, In: *General Ophthalmology*, Appleton & Lange,
15 Norwalk, CT, pp. 213-230 (1992)).

Most preferably, however, such drug administration will be accomplished by combining effective amounts of the agents of the invention with any of the sustained release ophthalmic delivery systems described by Davis, J.P. *et al.* (U.S. Patent No. 5,192,535, herein incorporated by reference).

20 Such preferred sustained release topical ophthalmic medicament delivery systems comprise an aqueous suspension at a pH of from about 3 to about 6.5 and an osmotic pressure of from about 10 to about 400 mOsm containing from about 0.1% to about 6.5% by weight, based on the total weight of the suspension, of a carboxyl-containing polymer prepared by polymerizing
25 one or more carboxyl-containing monoethylenically unsaturated monomers and less than about 5% by weight of a crosslinking agent, such weight percentages of monomers being based on the total weight of monomers polymerized. Desirably the polymer is prepared by suspension or emulsion polymerizing the monomer with the crosslinking agent to a particle size of not
30 more than about 50 μm , preferably not more than about 30 μm , in equivalent spherical diameter. The suspension has an initial viscosity of from about 1,000 to about 30,000 centipoises (cp) and is administrable to the eye in drop form at that initial viscosity. The polymer has average particle size of not more than about 50 μm , preferably not more than about 30 μm , in equivalent spherical
35 diameter. In general, such polymers will range in molecular weight estimated

to be about 250,000 to about 4,000,000, and preferably about 500,000 to about 2,000,000.

5 Aqueous suspensions containing polymer particles prepared by suspension or emulsion polymerization whose average dry particle size is appreciably larger than about 50 μm in equivalent spherical diameter are less comfortable when administered to the eye than suspensions otherwise identical in composition containing polymer particles whose equivalent spherical diameters are, on the average, below about 50 μm . Moreover, above the average 50 μm size, the advantage of substantially increased viscosity after
10 administration is not realized.

The lightly crosslinked suspension is administrable in drop form, upon contact of the lower pH suspension with the higher pH tear fluid of the eye, the suspension is rapidly gellable to a substantially greater viscosity than the viscosity of the suspension as originally administered in drop form.
15 Accordingly, the resulting more viscous gel can remain in the eye for a prolonged period of time so as to release

The polymer of the preferred intra-ocular drug delivery system is preferably prepared from at least about 50% by weight, more preferably at least about 90% by weight, of one or more carboxyl-containing
20 monoethylenically unsaturated monomers. Acrylic acid is the preferred carboxyl-containing, monoethylenically unsaturated monomer, but other unsaturated, polymerizable carboxyl-containing monomers, such as methacrylic acid, ethacrylic acid, b-methylacrylic acid (crotonic acid), cis-a-methylcrotonic acid (angelic acid), trans-a-methylcrotonic acid (tiglic acid), a-butylcrotonic acid, a-phenylacrylic acid, a-benzylacrylic acid, a-cyclohexylacrylic acid, b-phenylacrylic acid (cinnamic acid), coumaric acid (o-hydroxycinnamic acid), p-hydroxycoumaric acid (umbellic acid), and the like
25 can be used in addition to or instead of acrylic acid.

Such polymers are crosslinked by using a small percentage, i.e., less
30 than about 5%, such as from about 0.5% or from about 0.1% to about 5%, and preferably from about 0.2% to about 1%, based on the total weight of monomers present, of a polyfunctional crosslinking agent. The crosslinking agents of such compositions include non-polyalkenyl polyether difunctional crosslinking monomers such as divinyl glycol; 2,3-dihydroxyhexa-1,5-diene;
35 2,5-dimethyl-1,5-hexadiene; divinylbenzene; N,N-diallylacrylamide; N,N-diallylmethacrylamide and the like. A preferred crosslinking agent is divinyl

- 30 -

glycol. Also included are polyalkenyl polyether crosslinking agents containing two or more alkenyl ether groupings per molecule, preferably alkenyl ether groupings containing terminal $\text{H}_2\text{C}=\text{C}<$ groups, prepared by etherifying a polyhydric alcohol containing at least four carbon atoms and at least three hydroxyl groups with an alkenyl halide such as allyl bromide or the like, e.g., polyallyl sucrose, polyallyl pentaerythritol, or the like; see, e.g., Brown, U.S. Patent No. 2,798,053. Diolefinic non-hydrophilic macromeric crosslinking agents having molecular weights of from about 400 to about 8,000, such as insoluble di- and polyacrylates and methacrylates of diols and polyols, diisocyanate-hydroxyalkyl acrylate or methacrylate reaction products, and reaction products of isocyanate terminated prepolymers derived from polyester diols, polyether diols or polysiloxane diols with hydroxyalkyl-methacrylates, and the like, can also be used as the crosslinking agents; see, e.g., Mueller et al. U.S. Patents Nos. 4,192,827 and 4,136,250.

In a preferred method of preparing sustained release topical ophthalmic delivery systems, the foregoing suspensions are prepared and packaged at the desired viscosity of from 1,000 to about 30,000 centipoises, for administration to the eye in drop form. In a preferred delivery method, the foregoing suspensions, containing the medicament, are administered to the eye at the initial viscosity in drop form to cause the administered suspension, upon contact with the higher pH tear fluid of the eye, to rapidly gel *in situ* to a substantially greater viscosity than the viscosity of the suspension as originally administered in drop form. The more viscous gel remains in the eye for a prolonged period of time so as to release the medicament, entrapped in the more viscous gel formed in the eye, in sustained fashion.

It may be desirable to replace up to about 40% by weight of the carboxyl-containing monoethylenically unsaturated monomers by one or more non-carboxyl-containing monoethylenically unsaturated monomers containing only physiologically and ophthalmologically innocuous substituents.

The desired osmotic pressure is preferably achieved by using a physiologically and ophthalmologically acceptable salt in an amount of from about 0.01% to about 1% by weight, based on the total weight of the suspensions. A preferred salt is sodium chloride.

Generally, the dosage needed to provide an effective amount of the composition will vary depending upon such factors as the recipient's age,

- 31 -

condition, sex, and extent of disease, if any, and other variables, and can be adjusted and determined by one of ordinary skill in the art. Effective amounts of the compositions of the invention can vary from 0.01-1,000 mg/ml per dose or application, although lesser or greater amounts can be used. For
5 ophthalmic suspensions, the effective amounts will preferably be from about 0.005% to about 10% by weight, and most preferably from about 0.01% to about 5% by weight, based on the total weight of the suspension.

Having now generally described the invention, the same will be more readily understood through reference to the following examples which are
10 provided by way of illustration, and are not intended to be limiting of the present invention, unless specified.

EXAMPLE 1 CLONING OF TIGR cDNA

In order to clone the major DEX-inducible cDNA of HTM cells, a subtraction screening procedure was employed. In such subtractive screening
15 methods, cDNA molecules created from a complete population of cells are permitted to hybridize with a cDNA library constructed from RNA of different subpopulations of cells in order to identify clones that exhibit differential expression, and that thus reflect mRNA molecules that are induced or repressed in each population (Lamar, E.E. *et al.*, *Cell* 37:171-177 (1984);
20 Rubenstein, J.L.R. *et al.*, *Nucleic Acids Res.* 18:4833-4842 (1990); Hedrik, S.M. *et al.*, *Science* 308:149-153 (1984); Duguid, J.R. *et al.*, *Proc. Natl. Acad. Sci. (U.S.A.)* 85:5738-5742 (1988); Weiland, I. *et al.*, *Proc. Natl. Acad. Sci. (U.S.A.)* 87:2720-2724 (1990)).

cDNA was therefore prepared from mRNA of human trabecular meshwork (HTM) cells that had been incubated in 100 nM dexamethasone for 10 days, as well as from mRNA of untreated HTM cells. The cDNA library was constructed in lambda ZapII using strain XL-1 (Stratagene, San Diego). Approximately 30-50 µg of mRNA were obtained from 5×10^7 dexamethasone
25 treated cells as described by Nguyen, T.D. *et al.* (In: "Schriftenreihe der Akademie der Wissenschaften und der Literatur, Mainz," 331-343 (1993), herein incorporated by reference).
30

Two independent screenings of 20,000 phages each were conducted using a differential screening approach. Phages were separately probed with
35 labeled cDNA made from mRNA of untreated cells and of dexamethasone-

treated cells for 1 day and 10 days. Clones that exhibited an inducible response (i.e. increased labeling when probed with the dexamethasone treated cDNA relative to control cDNA) were desired candidates for further analysis.

5 Several cDNA clones were obtained that corresponded to mRNA produced in higher amounts in dexamethasone-treated cells. One cDNA clone, corresponding to mRNA that was present in the dexamethasone-treated cells, but absent from the untreated cells, was designated "clone II.2" or "TIGR" (T.D. Nguyen *et al.*, *Invest. Ophthalmol. Vis. Sci.* 32:789 (1991)). A second clone encoded alpha-1 antichymotrypsin.

10 The level of these changes was quantitated by both dot-blot and PCR methodology. In the dot-blot analysis, the DNA of the clones are serially diluted and applied to membranes which are then hybridized to labeled total cDNA of control, 1 day or 10 day dexamethasone-treated HTM cells. The dot-blot analysis revealed that the TIGR mRNA was the major induced species,
15 comprising 3-4% of the total cellular mRNA at day 10. An insignificant level of TIGR mRNA was detected in the control. The time course of dexamethasone treatment for days 2, 4, 7 and 10 revealed that TIGR was progressively induced (T.D. Nguyen *et al.*, *Invest. Ophthalmol. Vis. Sci.* 32:789 (1991)). Cycloheximide studies showed that the induction required protein
20 synthesis. Southern analysis and in-situ hybridization show multiple copy numbers of the gene. For PCR amplification analysis, the serial dilution PCR method (Chelly, J.D. *et al.*, *Eur. J. Biochem.* 187:691-698 (1990); Murphy, L.D. *et al.*, *Biochem.* 29:10350-10356 (1990); Singer-Sam, J.O. *et al.*, *Nucl. Acids Res.* 18:1255-1259 (1990)) was modified to maintain the exponential range of the
25 amplification throughout the quantitation procedure to measure and confirm the major and progressive induction of TIGR mRNA over a 10 day dexamethasone induction period. Quantitative PCR analysis revealed an induction level of about 20 fold compared to the level found in cells that had been treated with dexamethasone for only 1 day.

30 Northern analysis showed clone TIGR to be approximately 2.5 kb, and to encode a protein of unique sequence. The induction of the mRNA required protein synthesis and insulin-like growth factor reduced the induction effect by 50%. The TIGR mRNA induction was not observed in dexamethasone treated fibroblasts, keratinocytes, or ciliary epithelial cells. The pattern of
35 induction in HTM cells was distinguishable from other steroid induced proteins such as metallothionein, alpha1-acid glycoprotein, and TAT which

- 33 -

were maximally induced by one day dexamethasone treatment. In addition to dexamethasone, the TIGR mRNA was induced in HTM cells exposed to hydrogen peroxide, TPA, or glucose for 3-24 hours. Dexamethasone treatment produced substantial loss in the mRNAs for glucocorticoid receptors and heat shock proteins (e.g., hsp 90 mRNA levels fell approximately 20 fold after 10 days of dexamethasone treatment).

EXAMPLE 2

EXPRESSION OF TIGR

An ability to express substantial amounts of TIGR would facilitate the use of this protein for functional assays, and the development of anti-TIGR antibodies. To achieve such enhanced expression, the PVL1393 baculovirus transfer vector of Invitrogen Corp. was employed. A 2 Kb Eco-R1 fragment of the TIGR cDNA was inserted into the Eco R1 cloning site of PVL1393. PCR and sequencing analysis showed that the insert had been ligated in the correct orientation into the vector's polyhedrin promoter. Cotransfection of this construct and wild type baculovirus DNA into Sf9 insect cells produced high titers of recombinant protein. The Sf9 insect cell line can be obtained from the American Type Culture Collection, Rockville, MD, US, as deposit accession number ATCC CRL 1711. Methods of using such vectors and cells are described by Summers M.D. *et al.* (In: *A Manual of Methods for Baculovirus Vectors and Insect Cell Culture Procedures*, Texas Agricultural Experiment Station Bulletin No. 1555 (1987)), and Summers, M.D. (U.S. Patent 5,278,050).

PCR verification of these recombinants showed positive signal for the expressed gene. SDS gel analysis of the SF9 transfected cellular proteins showed that the new product had a molecular weight of about 55 KDa and amounted to 90-95% of the total protein produced. These values correlated well with the size of the HTM cell protein induced by dexamethasone (DEX) (Polansky, J.R. *et al.*, *Prog Clin Biol Res* 312:113-138, 1989) and the calculated MW from the cDNA sequence isolated. G-150 (Pharmacia) column purification and Edman degradation sequencing of the protein confirmed the open reading frame of the TIGR cDNA.

Studies of the recombinant protein thus suggested (1) that the 55 kD protein existed both in cells and in the medium, (2) that it underwent oligomerization, (3) phosphorylation, (4) glycosylation, (5) that it was susceptible to metalloprotease, (6) that it exhibited high affinity binding to

- 34 -

extracellular matrix and human trabecular meshwork cells, (7) that it exhibited progressive inductions with time in both cell and organ cultures, and (8) that it exhibited high expression in the HTM of glaucomatous patients as compared to normal patients. Significantly, the induction correlated with topical glucocorticoid effects on intra-ocular pressure in patients, and differed from other known glucocorticoid induction patterns which exhibit close to maximal induction at only one day of dexamethasone treatment.

EXAMPLE 3

STRUCTURAL CHARACTERISTICS OF TIGR

Clone TIGR was sequenced, and found to comprise a 2.0 kb cDNA molecule (SEQ ID NO:2). The full-length transcript was nearly 2.5 kb as determined by Northern analysis. The cDNA included two ATG start sites which produced two 55 kD proteins in both HTM and Sf9 cells. The larger protein was 497 amino acids, and is defined by the TIGR open reading frame (SEQ ID NO:1). The larger protein is due to the unprocessed form of TIGR; the smaller protein reflects the proteolytic cleavage of the TIGR signal peptide. The amino terminal sequence of these proteins has been verified by amino acid sequencing analysis. The post-translational modification of these proteins also produced a highly glycosylated TIGR form of about 66 kD.

Structural analysis of the clone demonstrated it encoded a novel extracellular protein of about 55 kD with an N-glycosylation site at SEQ ID NO:1 residues 57-60 and O-glycosylation sites at SEQ ID NO:1 residues 221-222; 222-223; 270-272; 305-306; 397-401; 453-457; 457-459, heparin sulfate binding (SEQ ID NO:1 residues 110-113 and 146-150) and initiation domains (SEQ ID NO:1 residues 223-224, 231-232 and 324-325), 7 consensus leucine zipper units, forming two stretches, one located at SEQ ID NO:1, residues 85-92 and 92-99, and five located at SEQ ID NO:1, residues 121-128; 128-135; 135-142; 142-149; and 149-156), and a potential GIP (guanidyl inositol phosphate) linkage. The 55 kD recombinant protein forms dimer or heteromer in the HTM medium as demonstrated by crosslinking studies, and it could self-aggregate. The recombinant protein had a specific ability to bind trabecular meshwork cells (4.3×10^{-9} M and 2.3×10^{-8} M) as shown by Skatchard analysis. In contrast, the protein showed non-saturable and low affinity binding ability for fibroblasts. The recombinant protein was shown to be a substrate for the 72 kD metalloprotease.

- 35 -

5 The anti-TIGR antibodies recognize a 66 kD protein in DEX-treated HTM medium. This protein was shown to be a highly glycosylated form of the 55 kD TIGR protein. This conclusion was supported by the observation that expression conducted in the presence of tunicamycin shifted production from 66 kD to 55 kD. The 66 kD glycosylated form of TIGR appears to be a hyaluronate binding protein, since it was found to be capable of binding to hyaluronic acid beads. Such binding proteins are defined by their ability to bind to such beads and to be eluted from the beads in the presence of 4 M guanidine after 0.15 and 1.5 M NaCl washes.

10 While the invention has been described in connection with specific embodiments thereof, it will be understood that it is capable of further modifications and this application is intended to cover any variations, uses, or adaptations of the invention following, in general, the principles of the invention and including such departures from the present disclosure as come
15 within known or customary practice within the art to which the invention pertains and as may be applied to the essential features hereinbefore set forth and as follows in the scope of the appended claims.

- 36 -

SEQUENCE LISTING

- 5 (1) GENERAL INFORMATION:
- (i) APPLICANT: NGUYEN, THAI D.
POLANSKY, JON R.
HUANG, WEIDONG
- 10 (ii) TITLE OF INVENTION: METHODS FOR THE DIAGNOSIS
OF GLAUCOMA
- (iii) NUMBER OF SEQUENCES: 3
- 15 (iv) CORRESPONDENCE ADDRESS:
(A) ADDRESSEE: HOWREY & SIMON
(B) STREET: 1299 PENNSYLVANIA AVE., N.W.
(C) CITY: WASHINGTON
(D) STATE: D.C.
20 (E) COUNTRY: US
(F) ZIP: 20004
- (v) COMPUTER READABLE FORM:
(A) MEDIUM TYPE: Floppy disk
25 (B) COMPUTER: IBM PC compatible
(C) OPERATING SYSTEM: PC-DOS/MS-DOS
(D) SOFTWARE: PatentIn Release #1.0, Version #1.25
- (vi) CURRENT APPLICATION DATA:
30 (A) APPLICATION NUMBER: US
(B) FILING DATE:
(C) CLASSIFICATION:
- (viii) ATTORNEY/AGENT INFORMATION:
35 (A) NAME: AUERBACH, JEFFREY I
(B) REGISTRATION NUMBER: 32,680
- (ix) TELECOMMUNICATION INFORMATION:
40 (A) TELEPHONE: (202) 383-7451
(B) TELEFAX: (202) 383-6610

- 37 -

(2) INFORMATION FOR SEQ ID NO:1:

(i) SEQUENCE CHARACTERISTICS:

5

(A) LENGTH: 497 amino acids

(B) TYPE: amino acid

(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: protein

10

(iii) HYPOTHETICAL: NO

(vii) IMMEDIATE SOURCE:

(B) CLONE: TIGR

15

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:1:

	Met	Arg	Phe	Phe	Cys	Ala	Arg	Cys	Cys	Ser	Phe	Gly	Pro	Glu	Met	Pro	
	1				5					10					15		
20	Ala	Val	Gln	Leu	Leu	Leu	Ala	Cys	Leu	Val	Trp	Asp	Val	Gly	Ala		
			20					25					30				
	Arg	Thr	Ala	Gln	Leu	Arg	Lys	Ala	Asn	Asp	Gln	Ser	Gly	Arg	Cys	Gln	
25			35				40						45				
	Tyr	Thr	Phe	Ser	Val	Ala	Ser	Pro	Asn	Glu	Ser	Ser	Cys	Pro	Glu	Gln	
		50					55					60					
30	Ser	Gln	Ala	Met	Ser	Val	Ile	His	Asn	Leu	Gln	Arg	Asp	Ser	Ser	Thr	
	65					70					75					80	
	Gln	Arg	Leu	Asp	Leu	Glu	Ala	Thr	Lys	Ala	Arg	Leu	Ser	Ser	Leu	Glu	
				85					90						95		
35	Ser	Leu	Leu	His	Gln	Leu	Thr	Leu	Asp	Gln	Ala	Ala	Arg	Pro	Arg	Arg	
			100						105					110			
	Pro	Arg	Arg	Arg	Glu	Arg	Asp	Gln	Leu	Glu	Thr	Gln	Thr	Arg	Glu	Leu	
40			115					120					125				
	Glu	Thr	Ala	Tyr	Ser	Asn	Leu	Leu	Arg	Asp	Lys	Ser	Val	Leu	Glu	Glu	
		130				135						140					
45	Glu	Lys	Lys	Arg	Leu	Arg	Gln	Glu	Asn	Glu	Asn	Leu	Ala	Arg	Arg	Leu	
	145				150					155						160	
	Glu	Ser	Ser	Ser	Gln	Glu	Val	Ala	Arg	Leu	Arg	Arg	Gly	Gln	Cys	Pro	
				165						170					175		
50	Ser	Thr	Arg	Asp	Thr	Ala	Arg	Ala	Val	Pro	Pro	Gly	Ser	Arg	Glu	Val	
				180					185						190		

- 38 -

Ser Thr Trp Asn Leu Asp Thr Leu Ala Phe Gln Glu Leu Lys Ser Glu
 195 200 205
 5 Leu Thr Glu Val Pro Ala Ser Arg Ile Leu Lys Glu Ser Pro Ser Gly
 210 215 220
 Tyr Leu Arg Ser Leu Arg Ser Gly Glu Gly Asp Thr Gly Cys Gly Glu
 225 230 235 240
 10 Leu Val Trp Val Gly Glu Pro Leu Thr Leu Arg Thr Ala Glu Thr Ile
 245 250 255
 Thr Gly Lys Tyr Gly Val Trp Met Arg Asp Pro Lys Pro Thr Tyr Pro
 260 265 270
 15 Tyr Ile Pro Gly Asp His Val Glu Asn Arg His Ser Trp His Gly Cys
 275 280 285
 Pro Pro Val Phe Glu Tyr Asp Leu Ile Ser Gln Phe Met Gln Gly Tyr
 290 295 300
 20 Pro Ser Lys Val His Ile Leu Pro Arg Pro Leu Glu Ser Thr Gly Arg
 305 310 315 320
 25 Val Val Tyr Ser Gly Ser Leu Tyr Phe Gln Gly Ala Glu Ser Arg Thr
 325 330 335
 Val Ile Arg Tyr Glu Leu Asn Thr Glu Thr Val Lys Ala Glu Lys Glu
 340 345 350
 30 Ile Pro Gly Ala Gly Tyr His Gly Gln Phe Pro Tyr Ser Trp Gly Gly
 355 360 365
 Tyr Thr Asp Ile Asp Leu Ala Val Asp Glu Ala Gly Leu Trp Val Ile
 370 375 380
 35 Tyr Ser Thr Asp Glu Ala Lys Gly Ala Ile Val Leu Ser Lys Leu Asn
 385 390 395 400
 40 Pro Glu Asn Leu Glu Leu Glu Gln Thr Trp Glu Thr Asn Ile Arg Lys
 405 410 415
 Gln Ser Val Ala Asn Ala Phe Ile Ile Cys Gly Thr Leu Tyr Thr Val
 420 425 430
 45 Ser Ser Tyr Thr Ser Ala Asp Ala Thr Val Asn Phe Ala Tyr Asp Thr
 435 440 445
 Gly Thr Gly Ile Ser Lys Thr Leu Thr Ile Pro Phe Lys Asn Arg Tyr
 450 455 460
 50 Lys Tyr Ser Ser Met Ile Asp Tyr Asn Pro Leu Glu Lys Lys Leu Phe
 465 470 475 480
 55 Ala Trp Asp Asn Leu Asn Met Val Thr Tyr Asp Ile Lys Leu Ser Lys
 485 490 495

Met

- 39 -

(2) INFORMATION FOR SEQ ID NO:2:

(i) SEQUENCE CHARACTERISTICS:

- 5 (A) LENGTH: 1968 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: double
(D) TOPOLOGY: linear

10 (ii) MOLECULE TYPE: cDNA

(iii) HYPOTHETICAL: NO

15 (iv) ANTI-SENSE: NO

(vii) IMMEDIATE SOURCE:

(B) CLONE: IL.2

20

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:2:

TTTTNAAGTN CATATCGAAT TCGGNACGAG GNAGAGCTTT CCAGAGGAAG CCTCACCAAG	60
25 CCTCTGCAAT GAGGTTCCTC TGTGCACGTT GCTGCAGCTT TGGGCCTGAG ATGCCAGCTG	120
TCCAGCTGCT GCTTCTGGCC TGCCTGGTGT GGGATGTGGG GGCCAGGACA GCTCAGCTCA	180
30 GGAAGGCCAA TGACCAGAGT GGCCGATGCC AGTATACCTT CAGTGTGGCC AGTCCCAATG	240
AATCCAGCTG CCCAGAGCAG AGCCAGGCCA TGTCACTCAT CCATAACTTA CAGAGAGACA	300
GCAOCACCCA ACGCTTAGAC CTGGAGGCCA CCAAAGCTCG ACTCAGCTCC CTGGAGAGCC	360
35 TCCTCCACCA ATTGACCTTG GACCAGGCTG CCAGGCCAG GAGACCCAGG AGGCGGGAGC	420
GGGACCAGCT GGAAACCCAA ACCAGAGAGT TGGAGACTGC CTACAGCAAC CTCCTCCGAG	480
40 ACAAGTCAGT TCTGGAGGAA GAGAAGAAGC GACTAAGGCA AGAAAATGAG AATCTGGCCA	540
GGAGGTTGGA AAGCAGCAGC CAGGAGGTAG CAAGGCTGAG AAGGGGCCAG TGTCCAGTA	600
CCCGAGACAC TGCTCGGGCT GTGCCACCAG GCTCCAGAGA AGTTTCTACG TGGAAATTGG	660
45 ACACTTTGGC CTTCAGGAA CTGAAGTCCG AGCTAACTGA AGTTCCTGCT TCCCGAATTT	720
TGAAGGAGAG CCCATCTGGC TATCTCAGGA GTCTCAGGAG TGGAGAGGGA GACACCGGAT	780
50 GTGGAGAACT AGTTTGGGTA GGAGAGCCTC TCACGCTGAG AACAGCAGAA ACAATTACTG	840
GCAAGTATGG TGTGTGATG CGAGACCCCA AGCCACCTA CCCCTACATC CCAGGAGACC	900
ACGTGGAGAA TCGACACAGT TGGCAGGAT GTCCGCCAGT TTTTGAGTAT GACCTCATCA	960
55 GCCAGTTTAT GCAGGGCTAC CCTTCTAAGG TTCACATACT GCCTAGGCCA CTGGAAAGCA	1020

- 40 -

	CGGGTCGTGT GGTGTACTCG GGGAGCCTCT ATTTCCAGGG CGCTGAGTCC AGAACTGTCA	1080
	TAAGATATGA GCTGAATACC GAGACAGTGA AGGCTGAGAA GGAAATCCCT GGAGCTGGCT	1140
5	ACCACGGACA GTTCCCGTAT TCTTGGGGTG GCTACACGGA CATTGACTTG GCTGTGGATG	1200
	AAGCAGGCCT CTGGGTCATT TACAGCACCG ATGAGGCCAA AGGTGCCATT GTCCTCTCCA	1260
10	AACTGAACCC AGAGAATCTG GAACTCGAAC AAACCTGGGA GACAAACATC CGTAAGCAGT	1320
	CAGTCGCCAA TGCCTTCATC ATCTGTGGCA CCTTGTACAC CGTCAGCAGC TACACCTCAG	1380
	CAGATGCTAC CGTCAACTTT GCTTATGACA CAGGCACAGG TATCAGCAAG ACCCTGACCA	1440
15	TCCCATTCAA GAACCGCTAT AAGTACAGCA GCATGATTGA CTACAACCCC CTGGAGAAGA	1500
	AGCTCTTTGC CTGGGACAAC TTGAACATGG TCACTTATGA CATCAAGCTC TCCAAGATGT	1560
20	GAAAAGCCTC CAAGCTGTAC AGGCAATGOC AGAAGGAGAT GCTCAGGGCT CCTGGGGGGA	1620
	GAGGGCTGAA GGGAGAGCCA GCCAGCCAGG GCCCAGGAGC TTTGACTGCT TTCCAAGTTT	1680
	TCATTAATCC AGAAGGATGA ACATGGTCAC CATCTAACTA TTCAGGAATT GTAGTCTGAG	1740
25	GGCGTAGACC ATTTCATATA ATAAATATCC TTTATCTTCT GTCAGCATTT ATGGGATGTT	1800
	TAATGACATA GTTCAAGTTT TTCTGAAAA CCATTGCTCT TGCATGTTAC ATGGTTACCA	1860
30	CAAGCCACAA TAAAAAGCAT AACTTCTAAA GGAAGCAGAA TAGCTCCTCT GCCCAGCATC	1920
	GAATATAAGT AAGATGCATT TACTACAGTT GGCTTCTAAT GCTTCAGA	1968

(2) INFORMATION FOR SEQ ID NO:3:

35 (i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 1491 base pairs
 (B) TYPE: nucleic acid
 (C) STRANDEDNESS: double
 (D) TOPOLOGY: linear

40

(ii) MOLECULE TYPE: cDNA

(iii) HYPOTHETICAL: NO

45

(iv) ANTI-SENSE: NO

(vii) IMMEDIATE SOURCE:

(B) CLONE: TIGR coding sequence

- 41 -

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:3:

5	ATGAGGTTCT TCTGTGCAG TTGCTGCAGC TTTGGGCTG AGATGCCAGC TGTCCAGCTG	60
	CTGCTTCTGG CCTGCCGTGT GTGGGATGTG GGGGCCAGGA CAGCTCAGCT CAGGAAGGCC	120
	AATGACCAGA GTGGCCGATG CCAGTATACC TTCAGTGTGG CCAGTCCCAA TGAATCCAGC	180
10	TGCCCAGAGC AGAGCCAGGC CATGTTCAGTC ATCCATAACT TACAGAGAGA CAGCAGCACC	240
	CAACGCTTAG ACCTGGAGGC CACCAAAGCT CGACTCAGCT CCCTGGAGAG CCTCCTCCAC	300
15	CAATTGACCT TGGACCAGGC TGCAGGCCCC AGGAGACCCA GGAGGCGGA GCGGGACCAG	360
	CTGGAAACCC AAACAGAGA GTTGGAGACT GCCTACAGCA ACCTCCTCCG AGACAAGTCA	420
	GTTCGTGAGG AAGAGAAGAA GCGACTAAGG CAAGAAAATG AGAATCTGGC CAGGAGGTTG	480
20	GAAAGCAGCA GCCAGGAGGT AGCAAGGCTG AGAAGGGGCC AGTGTCCCAG TACCCGAGAC	540
	ACTGCTCGGG CTGTGCCACC AGGCTCCAGA GAAGTTTCTA CGTGAATTTT GGACACTTTG	600
25	GCCTTCCAGG AACTGAAGTC CGAGCTAACT GAAGTTCCTG CTTCGCCAAT TTTGAAGGAG	660
	AGCCCATCTG GCTATCTCAG GAGTCTCAGG AGTGGAGAGG GAGACACCGG ATGTGGAGAA	720
	CTAGTTTGGG TAGGAGAGCC TCTCAGCTG AGAACAGCAG AAACAATTAC TGGCAAGTAT	780
30	GGTGTGTGGA TCGGAGACCC CAAGCCACCC TACCCCTACA TCCAGGAGA CCACGTGGAG	840
	AATCGACACA GTTGGCACGG ATGTCCGCCA GTTTTGTAGT ATGACCTCAT CAGCAGTTT	900
35	ATGCAGGGCT ACCCTTCTAA GGTTTCACATA CTGCCTAGGC CACTGGAAAG CACGGGTCGT	960
	GTGGTGTACT CGGGGAGCCT CTATTTCCAG GGCCTGAGT CCAGAACTGT CATAAGATAT	1020
	GAGCTGAATA CCGAGACAGT GAAAGCTGAG AAGGAAATCC CTGGAGCTGG CTACCACGGA	1080
40	CAGTTCCCGT ATTCTTGGGG TGGCTACAGG GACATTGACT TGGCTGTGGA TGAAGCAGGC	1140
	CTCTGGGTCA TTTACAGCAC CGATGAGGCC AAAGGTGCCA TTGTCTCTTC CAAACTGAAC	1200
45	CCAGAGAATC TGGAACTCGA ACAAACTGG GAGACAAACA TCCGTAAGCA GTCAGTCGCC	1260
	AATGCCTTCA TCATCTGTGG CACCTTGATC ACCGTCAGCA GCTACACCTC AGCAGATGCT	1320
	ACCGTCAACT TTGCTTATGA CACAGGCACA GGTATCAGCA AGACCCCTGAC CATCCCATTC	1380
50	AAGAACCCTG ATAAGTACAG CAGCATGATT GACTACAACC CCCTGGAGAA GAAGCTCTTT	1440
	GCCTGGGACA ACTTGAACAT GGTCACTTAT GACATCAAGC TCTCCAAGAT G	1491

- 42 -

WHAT IS CLAIMED IS:

1. A substantially purified TIGR protein having the sequence of SEQ ID NO:1 residues 1-497 or of SEQ ID NO:1 residues 15-497.
5
2. The substantially purified TIGR protein of claim 1, wherein said protein has the sequence of SEQ ID NO:1 residues 1-497.
3. The substantially purified TIGR protein of claim 1, wherein said
10 protein has the sequence of SEQ ID NO:1 residues 15-497.
4. A nucleic acid molecule that encodes a TIGR protein.
5. The nucleic acid molecule of claim 4 that comprises the sequence
15 of SEQ ID NO:3.
6. The nucleic acid molecule of claim 4 that comprises the sequence of SEQ ID NO:2.
7. An antibody capable of specifically binding to a TIGR protein.
20
8. The antibody of claim 7, wherein said antibody is detectably labeled.
9. A method for diagnosing glaucoma in a patient which comprises
25 determining whether the amount of a TIGR protein present in the trabecular meshwork of an eye of said patient exceeds the amount of that TIGR protein present in the trabecular meshwork of an eye of an individual who does not have glaucoma and is not predisposed to have glaucoma, wherein the
30 detection of an altered amount of said TIGR protein is indicative of glaucoma.

- 43 -

10. The method of claim 9, wherein said altered amount of said TIGR protein is an abnormally excessive amount.

5 11. The method of claim 9, wherein said altered amount of said TIGR protein is an abnormally diminished amount.

12. The method of claim 9, wherein said determination is accomplished by determining the amount of a TIGR mRNA present in cells of the trabecular meshwork.

10

13. The method of claim 9, wherein said determination is accomplished by administering an anti-TIGR antibody to said patient and determining the extent to which said antibody binds to the extracellular matrix of the trabecular meshwork.

15

14. The method of claim 9, wherein said determination is accomplished by administering a peptide that binds a TIGR protein of said patient and determining the extent to which said peptide binds to the extracellular matrix of the trabecular meshwork

20

15. A method of treating glaucoma which comprises administering to a glaucomatous patient an effective amount of an agent that inhibits the synthesis or binding ability of a TIGR protein.

25 16. The method of claim 15, wherein said agent inhibits the synthesis of said TIGR protein.

17. The method of claim 15, wherein said agent is a nucleic acid molecule that provides antisense repression of the TIGR gene.

30

- 44 -

18. The method of claim 15, wherein said agent is a nucleic acid molecule that provides triplex repression of the TIGR gene.

5 19. The method of claim 15, wherein said agent inhibits the binding ability of a TIGR protein.

20. The method of claim 15, wherein said agent is a protein that binds a TIGR protein.

10 21. The method of claim 20, wherein said protein is an anti-TIGR antibody.

22. The method of claim 15, wherein said agent is a peptide fragment of TIGR that binds TIGR.

15 23. A method for diagnosing glaucoma in a patient under evaluation for suspected glaucoma which comprises assaying the concentration of a molecule, whose concentration is dependent upon the expression of a TIGR gene, said molecule being present in a sample of cells or bodily fluid of said patient, in comparison to the concentration of that molecule present in a
20 sample of cells or bodily fluid of an individual who does not have glaucoma and is not predisposed to have glaucoma, wherein a concentration of said molecule differs from that found in said individual who does not have glaucoma and is not predisposed to have glaucoma is diagnostic of glaucoma in said patient.

25

24. The method of claim 23, wherein said bodily fluid is selected from the group consisting of glaucomatous cell extract, fluid from the anterior chamber of the eye, blood, lymph and serum.

- 45 -

25. The method of claim 23, wherein said molecule is an mRNA molecule encoded by said TIGR gene, or a cDNA molecule encoded by said TIGR gene.

5 26. The method of claim 25, wherein said concentration of said mRNA molecule is assayed by incubating a sample of said bodily fluid in the presence of a nucleic acid molecule that hybridizes to said mRNA molecule.

10 27. The method of claim 23, wherein said molecule is a protein molecule expressed by said TIGR gene.

15 28. The method of claim 23, wherein prior to performing said assay, said patient is provided with a TIGR gene-inducing amount of a glucocorticoid; and wherein said comparison is made between the concentration of said molecule in said sample of cells or bodily fluid of said patient, and the concentration of said molecule in a sample of cells or bodily fluid of an individual who does not have glaucoma and is not predisposed to have glaucoma, but who has also received said TIGR gene-inducing amount of a said glucocorticoid.

20 29. The method of claim 28, wherein said molecule is an mRNA molecule encoded by said TIGR gene, or a cDNA molecule encoded by said TIGR gene.

25 30. The method of claim 28, wherein said molecule is a protein molecule expressed by said TIGR gene.

30 31. The method of claim 27, wherein said expression of said TIGR gene is assayed by incubating a sample of said cells or said bodily fluid in the presence of antibodies elicited in response to immunization of an antibody-

- 46 -

producing cell with a recombinantly produced TIGR protein molecule, wherein said antibody molecule binds to said TIGR protein of said sample.

32. The method of claim 31, wherein said antibodies recognize a
5 TIGR protein molecule expressed by steroid-treated human trabecular cells.

33. A method for diagnosing glaucoma in a patient which comprises the steps:

(A) incubating under conditions permitting nucleic acid
10 hybridization: a marker nucleic acid molecule, said marker nucleic acid molecule comprising a nucleotide sequence of a polynucleotide that is linked to a TIGR gene, and a complementary nucleic acid molecule obtained from a cell or a bodily fluid of said patient, wherein nucleic acid hybridization between said marker nucleic acid molecule, and said complementary nucleic
15 acid molecule obtained from said patient permits the detection of a polymorphism whose presence is predictive of a mutation affecting TIGR Response in said patient;

(B) permitting hybridization between said TIGR-encoding marker nucleic acid molecule and said complementary nucleic acid molecule obtained
20 from said patient; and

(C) detecting the presence of said polymorphism, wherein the detection of said polymorphism is diagnostic of glaucoma.

34. The method of claim 33, wherein said TIGR Response affected by
25 said mutation is TIGR level.

35. The method of claim 33, wherein said TIGR Response affected by said mutation is TIGR pattern.

30 36. The method of claim 33, wherein said marker nucleic acid molecule comprises a nucleotide sequence of a polynucleotide that is

- 47 -

genetically linked to a TIGR gene, and has the sequence of a polynucleotide of chromosome 1, p or chromosome 12, p.

5 37. The method of claim 36, wherein said marker nucleic acid molecule has the sequence of a polynucleotide of chromosome 1, p34-p38 or chromosome 12, p11-p17.

10 38. The method of claim 37, wherein said marker nucleic acid molecule has the sequence of a polynucleotide of chromosome 1, p36 or chromosome 12, p13 or chromosome 12, q15.

15 39. The method of claim 33, wherein said marker nucleic acid molecule comprises a nucleotide sequence of a polynucleotide that is genetically linked to a TIGR gene, and has the sequence of a polynucleotide of chromosome 1, p or chromosome 11, p.

20 40. The method of claim 39, wherein said marker nucleic acid molecule has the sequence of a polynucleotide of chromosome 1, p34-p38 or chromosome 11, p11-p17.

 41. The method of claim 40, wherein said marker nucleic acid molecule has the sequence of a polynucleotide of chromosome 1, p36 or chromosome 11, p13 or chromosome 11, q15.

25 42. The method of claim 33, wherein said marker nucleic acid molecule comprises a nucleotide sequence of a polynucleotide that is genetically linked to a TIGR gene, and has the sequence of a polynucleotide of chromosome 1, p or chromosome 10, p.

- 48 -

43. The method of claim 42, wherein said marker nucleic acid molecule has the sequence of a polynucleotide of chromosome 1, p34-p38 or chromosome 10, p11-p17.

5 44. The method of claim 43, wherein said marker nucleic acid molecule has the sequence of a polynucleotide of chromosome 1, p36 or chromosome 10, p13 or chromosome 10, q15.

10 45. The method of claim 33, wherein said marker nucleic acid molecule comprises a nucleotide sequence of a polynucleotide that is physically linked to within 1 mb of a TIGR gene.

15 46. The method of claim 45, wherein said marker nucleic acid molecule comprises a nucleotide sequence of a polynucleotide that is physically linked to within 100 kb of a TIGR gene.

20 47. The method of claim 46, wherein said marker nucleic acid molecule comprises a nucleotide sequence of a polynucleotide that is physically linked to within 10 kb of a TIGR gene.

 48. The method of claim 33, wherein said marker nucleic acid molecule comprises a TIGR-encoding nucleic acid molecule, or fragment thereof.

25 49. The method of claim 33, wherein said bodily fluid is selected from the group consisting of glaucomatous cell extract, fluid from the anterior chamber of the eye, blood, lymph and serum.

30 50. The method of claim 33, wherein said complementary nucleic acid molecule obtained from a cell or present in a bodily fluid of said patient is expressed by steroid-treated cells.

- 49 -

51. The method of claim 50, wherein said bodily fluid is ocular aqueous, and wherein said cells are HTM cells.

5 52. A method for diagnosing glaucoma in a patient which comprises the steps:

- (A) obtaining a sample of cells or bodily fluid from said patient;
 - (B) determining whether said sample contains a protein whose presence is predictive of a mutation affecting TIGR Response in said patient;
- 10 wherein the detection of said protein is diagnostic for glaucoma.

53. A method for diagnosing steroid sensitivity in a patient under evaluation for suspected steroid sensitivity which comprises the steps:

- (A) obtaining a sample of cells or bodily fluid from said patient;
 - (B) assaying said sample to determine the concentration of a molecule, whose concentration is dependent upon the expression of a TIGR gene, in comparison to the concentration of that molecule present in a sample of cells or bodily fluid of an individual who does not have steroid sensitivity and is not predisposed to have steroid sensitivity from steroid sensitivity,
- 15 20 wherein a concentration of said molecule differs from that found in said individual who does not have steroid sensitivity and is not predisposed to have steroid sensitivity is diagnostic of steroid sensitivity in said patient.

54. The method of claim 53, wherein said bodily fluid is selected from the group consisting of fluid from the anterior chamber of the eye, blood, lymph and serum.

25

55. The method of claim 54, wherein said molecule is an mRNA molecule encoded by said TIGR gene, or a cDNA molecule encoded by said TIGR gene.

30

- 50 -

56. The method of claim 55, wherein said concentration of said mRNA molecule is assayed by incubating a sample of said bodily fluid in the presence of a nucleic acid molecule that hybridizes to said mRNA molecule.

5

57. The method of claim 53, wherein said molecule is a protein molecule expressed by said TIGR gene.

58. The method of claim 53, wherein prior to performing said assay, said patient is provided with a TIGR gene-inducing amount of a glucocorticoid; and wherein said comparison is made between the concentration of said molecule in said sample of cells or bodily fluid of said patient, and the concentration of said molecule in a sample of cells or bodily fluid of an individual who does not have steroid sensitivity and is not predisposed to have steroid sensitivity, but who has also received said TIGR gene-inducing amount of a said glucocorticoid.

10

15

59. A method for diagnosing steroid sensitivity in a patient which comprises the steps:

20

(A) incubating under conditions permitting nucleic acid hybridization: a marker nucleic acid molecule, said marker nucleic acid molecule comprising a nucleotide sequence of a polynucleotide that is linked to a TIGR gene, and a complementary nucleic acid molecule obtained from a cell or a bodily fluid of said patient, wherein nucleic acid hybridization between said marker nucleic acid molecule, and said complementary nucleic acid molecule obtained from said patient permits the detection of a polymorphism whose presence is predictive of a mutation affecting TIGR Response in said patient;

25

(B) permitting hybridization between said TIGR gene-linked nucleic acid molecule and said complementary nucleic acid molecule obtained from said patient; and

30

- 51 -

(C) detecting the presence of said polymorphism, wherein the detection of said polymorphism is diagnostic of steroid sensitivity.

5 60. The method of claim 59, wherein said TIGR Response affected by said mutation is TIGR level.

 61. The method of claim 59, wherein said TIGR Response affected by said mutation is TIGR pattern.

10 62. The method of claim 59, wherein said complementary nucleic acid molecule obtained from a cell or a bodily fluid of said patient has been amplified using a nucleic acid amplification method.

15 63. The method of claim 62, wherein said nucleic acid amplification method is polymerase chain amplification.

 64. The method of claim 62, wherein said nucleic acid amplification method is ligase chain reaction.

20 65. The method of claim 62, wherein said nucleic acid amplification method is oligonucleotide ligation assay.

 66. The method of claim 62, wherein said nucleic acid amplification method is allelic specific oligomers.

25 67. The method of claim 62, wherein said nucleic acid amplification method is branched DNA technology.

30 68. The method of claim 62, wherein said nucleic acid amplification method is transcription based amplification.

- 52 -

69. The method of claim 62, wherein said nucleic acid amplification method is iso thermal amplification.

5 70. The method of claim 33, wherein said complementary nucleic acid molecule obtained from a cell or a bodily fluid of said patient has been amplified using a nucleic acid amplification method.

10 71. The method of claim 70, wherein said nucleic acid amplification method is polymerase chain amplification.

 72. The method of claim 70, wherein said nucleic acid amplification method is ligase chain reaction.

15 73. The method of claim 70, wherein said nucleic acid amplification method is oligonucleotide ligation assay.

 74. The method of claim 70, wherein said nucleic acid amplification method is allelic specific oligomers.

20 75. The method of claim 70, wherein said nucleic acid amplification method is branched DNA technology.

 76. The method of claim 70, wherein said nucleic acid amplification method is transcription based amplification.

25 77. The method of claim 70, wherein said nucleic acid amplification method is iso thermal amplification.

30

1/4

Figure 1A

```

-1          -21          -41
T TTTNAAGTNC ATATCGAATT CGGNACGAGG NAGAGCTTTC CAGAGGAAGC CTC
. ....

-61          -81
ACCAAGC CTCTGCA ATG AGG TTC TTC TGT GCA CGT TGC TGC AGC TTT
..... met arg phe phe cys ala arg cys cys ser phe

-101         -121         -141
GGG CCT GAG ATG CCA GCT GTC CAG CTG CTG CTT CTG GCC TGC CTG
gly pro glu met pro ala val gln leu leu leu leu ala cys leu

-161         -181
GTG TGG GAT GTG GGG GCC AGG ACA GCT CAG CTC AGG AAG GCC AAT
val trp asp val gly ala arg thr ala gln leu arg lys ala asn

-201         -221
GAC CAG AGT GGC CGA TGC CAG TAT ACC TTC AGT GTG GCC AGT CCC
asp gln ser gly arg cys gln tyr thr phe ser val ala ser pro

-241         -261
AAT GAA TCC AGC TGC CCA GAG CAG AGC CAG GCC ATG TCA GTC ATC
asn glu ser ser cys pro glu gln ser gln ala met ser val ile

-281         -301         -321
CAT AAC TTA CAG AGA GAC AGC AGC ACC CAA CGC TTA GAC CTG GAG
his asn leu gln arg asp ser ser thr gln arg leu asp leu glu

-341         -361
GCC ACC AAA GCT CGA CTC AGC TCC CTG GAG AGC CTC CTC CAC CAA
ala thr lys ala arg leu ser ser leu glu ser leu leu his gln

-381         -401
TTG ACC TTG GAC CAG GCT GCC AGG CCC AGG AGA CCC AGG AGG CGG
leu thr leu asp gln ala ala arg pro arg arg pro arg arg arg

-421         -441
GAG CGG GAC CAG CTG GAA ACC CAA ACC AGA GAG TTG GAG ACT GCC
glu arg asp gln leu glu thr gln thr arg glu leu glu thr ala

```

Figure 1B

-461 -481 -501
TAC AGC AAC CTC CTC CGA GAC AAG TCA GTT CTG GAG GAA GAG AAG
tyr ser asn leu leu arg asp lys ser val leu glu glu glu lys

-521 -541
AAG CGA CTA AGG CAA GAA AAT GAG AAT CTG GCC AGG AGG TTG GAA
lys arg leu arg gln glu asn glu asn leu ala arg arg leu glu

-561 -581
AGC AGC AGC CAG GAG GTA GCA AGG CTG AGA AGG GGC CAG TGT CCC
ser ser ser gln glu val ala arg leu arg arg gly gln cys pro

-601 -621
AGT ACC CGA GAC ACT GCT CGG GCT GTG CCA CCA GGC TCC AGA GAA
ser thr arg asp thr ala arg ala val pro pro gly ser arg glu

-641 -661 -681
GTT TCT ACG TGG AAT TTG GAC ACT TTG GCC TTC CAG GAA CTG AAG
val ser thr trp asn leu asp thr leu ala phe gln glu leu lys

-701 -721
TCC GAG CTA ACT GAA GTT CCT GCT TCC CGA ATT TTG AAG GAG AGC
ser glu leu thr glu val pro ala ser arg ile leu lys glu ser

-741 -761
CCA TCT GGC TAT CTC AGG AGT CTC AGG AGT GGA GAG GGA GAC ACC
pro ser gly tyr leu arg ser leu arg ser gly glu gly asp thr

-781 -801
GGA TGT GGA GAA CTA GTT TGG GTA GGA GAG CCT CTC ACG CTG AGA
gly cys gly glu leu val trp val gly glu pro leu thr leu arg

-821 -841 -861
ACA GCA GAA ACA ATT ACT GGC AAG TAT GGT GTG TGG ATG CGA GAC
thr ala glu thr ile thr gly lys tyr gly val trp met arg asp

Figure 1C

-881 -901
 CCC AAG CCC ACC TAC CCC TAC ATC CCA GGA GAC CAC GTG GAG AAT
 pro lys pro thr tyr pro tyr ile pro gly asp his val glu asn

-921 -941
 CGA CAC AGT TGG CAC GGA TGT CCG CCA GTT TTT GAG TAT GAC CTC
 arg his ser trp his gly cys pro pro val phe glu tyr asp leu

-961 -981
 ATC AGC CAG TTT ATG CAG GGC TAC CCT TCT AAG GTT CAC ATA CTG
 ile ser gln phe met gln gly tyr pro ser lys val his ile leu

-1001 -1021 -1041
 CCT AGG CCA CTG GAA AGC ACG GGT CGT GTG GTG TAC TCG GGG AGC
 pro arg pro leu glu ser thr gly arg val val tyr ser gly ser

-1061 -1081
 CTC TAT TTC CAG GGC GCT GAG TCC AGA ACT GTC ATA AGA TAT GAG
 leu tyr phe gln gly ala glu ser arg thr val ile arg tyr glu

-1101 -1121
 CTG AAT ACC GAG ACA GTG AAG GCT GAG AAG GAA ATC CCT GGA GCT
 leu asn thr glu thr val lys ala glu lys glu ile pro gly ala

-1141 -1161
 GGC TAC CAC GGA CAG TTC CCG TAT TCT TGG GGT GGC TAC ACG GAC
 gly tyr his gly gln phe pro tyr ser trp gly gly tyr thr asp

-1181 -1201 -1221
 ATT GAC TTG GCT GTG GAT GAA GCA GGC CTC TGG GTC ATT TAC AGC
 ile asp leu ala val asp glu ala gly leu trp val ile tyr ser

-1241 -1261
 ACC GAT GAG GCC AAA GGT GCC ATT GTC CTC TCC AAA CTG AAC CCA
 thr asp glu ala lys gly ala ile val leu ser lys leu asn pro

-1281 -1301
 GAG AAT CTG GAA CTC GAA CAA ACC TGG GAG ACA AAC ATC CGT AAG
 glu asn leu glu leu glu gln thr trp glu thr asn ile arg lys

4/4

Figure 1D

-1321 -1341
CAG TCA GTC GCC AAT GCC TTC ATC ATC TGT GGC ACC TTG TAC ACC
gln ser val ala asn ala phe ile ile cys gly thr leu tyr thr

-1361 -1381 -1401
GTC AGC AGC TAC ACC TCA GCA GAT GCT ACC GTC AAC TTT GCT TAT
val ser ser tyr thr ser ala asp ala thr val asn phe ala tyr

-1421 -1441
GAC ACA GGC ACA GGT ATC AGC AAG ACC CTG ACC ATC CCA TTC AAG
asp thr gly thr gly ile ser lys thr leu thr ile pro phe lys

-1461 -1481
AAC CGC TAT AAG TAC AGC AGC ATG ATT GAC TAC AAC CCC CTG GAG
asn arg tyr lys tyr ser ser met ile asp tyr asn pro leu glu

-1501 -1521
AAG AAG CTC TTT GCC TGG GAC AAC TTG AAC ATG GTC ACT TAT GAC
lys lys leu phe ala trp asp asn leu asn met val thr tyr asp

-1541 -1561 -1581
ATC AAG CTC TCC AAG ATG TGA AAAGCCTCC AAGCTGTACA GGCAATGGCA
ile lys leu ser lys met

-1601 -1621
GAAGGAGATG CTCAGGGCTC CTGGGGGGAG CAGGGCTGAA GGGAGAGCCA

-1641 -1661
GCCAGCCAGC GCCCAGGAGC TTTGACTGCT TTCCAAG

-1681 -1701 -1721
TTT TCATTAATCC AGAAGGATGA ACATGGTCAC CATCTAACTA TTCAGGAATT G

-1741 -1761 -1781
TAGTCTGAG GGCCTAGACC ATTTCATATA ATAAATATCC TTTATCTTCT GTCAGC

-1801 -1821
ATTT ATGGGATGTT TAATGACATA GTTCAAGTTT TTCCTGAAAA CCATTGCTCT

-1841 -1861 -1881
TGCATGTTAC ATGGTTACCA CAAGCCACAA TAAAAGCAT AACTTCTAAA GGAAG

-1901 -1921 -1941
CAGAA TAGCTCCTCT GGCCAGCATC GAATATAAGT AAGATGCATT TACTACAGTT

-1961
GGCTTCTAAT GCTTCAGA

INTERNATIONAL SEARCH REPORT

International Application No

PCT/US 95/14024

A. CLASSIFICATION OF SUBJECT MATTER

IPC 6 C12N15/12 A61K39/395 C07K14/47 G01N33/68 G01N33/566
 G01N33/53 A61K38/17 A61K48/00 //C12Q1/68

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)

IPC 6 C07K C12N G01N A61K

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practical, search terms used)

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category *	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
A	INVEST. OPHTHALMOL. VIS. SCI., vol. 32, 1991 page 789 NGUYEN ET AL. 'Molecular biology studies of steroid-induced glaucoma model using cultured human trabecular meshwork' cited in the application see abstract nr 600-6:15 ---	1-6,9, 15,17, 23-30, 33,35, 49-51, 53-59, 61-63,76
A	INVEST. OPHTHALMOL. VIS. SCI., vol. 31, no. 4, 1990 page 505 NGUYEN ET AL. 'Cloning of glucocorticoid-induced proteins in HTM cells : verification of progressive, high dose dependent cDNAs to correlate with effects on IOP' see abstract nr 2479- 8:15 ---	1-6,9, 12, 23-26, 28,53, 54,58, 59,62, 63,70,71
-/--		

☒ Further documents are listed in the continuation of box C.☐ Patent family members are listed in annex.

* Special categories of cited documents :

- *A* document defining the general state of the art which is not considered to be of particular relevance
- *E* earlier document but published on or after the international filing date
- *L* document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)
- *O* document referring to an oral disclosure, use, exhibition or other means
- *P* document published prior to the international filing date but later than the priority date claimed

- *T* later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention
- *X* document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone
- *Y* document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art
- *A* document member of the same patent family

Date of the actual completion of the international search

11 March 1996

Date of mailing of the international search report

27.03.96

Name and mailing address of the ISA

European Patent Office, P.B. 5818 Patentlaan 2
 NL - 2280 HV Rijswijk
 Tel. (+ 31-70) 340-2040, Tx. 31 651 epo nl,
 Fax: (+ 31-70) 340-3016

Authorized officer

Gac, G

INTERNATIONAL SEARCH REPORT

Inter national Application No

PCT/US 95/14024

C(Continuation) DOCUMENTS CONSIDERED TO BE RELEVANT

Category *	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
A	<p>PROG. CLIN. BIOL. RES., vol. 312, 1989 pages 113-138, POLANSKY ET AL. 'The ocular effects of prostaglandins and other eicosanoids : Eicosanoid production and glucocorticoid regulatory mechanisms in cultured human trabecular meshwork cells' see pages 126-129, 132-134 see page 124</p> <p>---</p>	<p>1-3, 9-12, 23-30, 33-35, 50,51, 53-58, 70,71,76</p>
A	<p>INVEST. OPHTHALMOL. VIS. SCI., vol. 30, no. 8, August 1989 pages 1843-1847, PARTRIDGE ET AL. 'Dexamethazone induces specific proteins in human trabecular meshwork cells.' see the whole document</p> <p>---</p>	<p>1-3,53, 58,59</p>
A	<p>GLAUCOMA UPDATE IV, 1991 SPRINGER VERLAG , BERLIN, pages 20-29, POLANSKY ET AL. 'In-vitro correlates of glucocorticoid effects on intraocular pressure' cited in the application see the whole document</p> <p>-----</p>	<p>1-3,33, 53,58,59</p>

INTERNATIONAL SEARCH REPORT

International application No.

PCT/US 95/14024

Box I Observations where certain claims were found unsearchable (Continuation of item 1 of first sheet)

This international search report has not been established in respect of certain claims under Article 17(2)(a) for the following reasons:

1. ☒ Claims Nos.: 9-22, 28-30, 58
because they relate to subject matter not required to be searched by this Authority, namely:
Remark: Although these claims are directed to a method of treatment of diagnostic method practised on the human/animal body (Art. 17.2.a) and Rule 39.2.1v) PCT) the search has been carried out and based on the alleged effects of the compound/composition.
2. ☐ Claims Nos.:
because they relate to parts of the international application that do not comply with the prescribed requirements to such an extent that no meaningful international search can be carried out, specifically:
3. ☐ Claims Nos.:
because they are dependent claims and are not drafted in accordance with the second and third sentences of Rule 6.4(a).

Box II Observations where unity of invention is lacking (Continuation of item 2 of first sheet)

This International Searching Authority found multiple inventions in this international application, as follows:

1. ☐ As all required additional search fees were timely paid by the applicant, this international search report covers all searchable claims.
2. ☐ As all searchable claims could be searched without effort justifying an additional fee, this Authority did not invite payment of any additional fee.
3. ☐ As only some of the required additional search fees were timely paid by the applicant, this international search report covers only those claims for which fees were paid, specifically claims Nos.:
4. ☐ No required additional search fees were timely paid by the applicant. Consequently, this international search report is restricted to the invention first mentioned in the claims; it is covered by claims Nos.:

Remark on Protest

- ☐ The additional search fees were accompanied by the applicant's protest.
- ☐ No protest accompanied the payment of additional search fees.

THIS PAGE BLANK (USPTO)